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10/631, 958

(FILE 'HOME' ENTERED AT 12:22:25 ON 13 SEP 2005)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 12:22:51 ON 13 SEP 2005

L1 181 S CERAMIDE (W)KINASE?  
L2 99 S HUMAN AND L1  
L3 7258991 S CLON? OR EXPRESS? OR RECOMBINANT  
L4 49 S L2 AND L3  
L5 22 DUP REM L4 (27 DUPLICATES REMOVED)  
L6 2 S SHINGOSINE (W)KINASE?  
L7 2026 S SPHINGOSINE (W)KINASE?  
L8 1467318 S HOMOLOG? OR IDENTIT?  
L9 526525 S HUMAN AND L8  
L10 99 S L7 AND L9  
L11 47 DUP REM L10 (52 DUPLICATES REMOVED)  
E KOSSIDA S/AU  
L12 72 S E3-E4  
E ENICAS J/AU  
E ENCINAS J/AU  
L13 27 S E3  
E TAKAO E/AU  
L14 19 S E3  
L15 117 S L12 OR L13 OR L14  
L16 1 S L2 AND L15

=>

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=> s ceramide (w)kinase?  
L1 181 CERAMIDE (W) KINASE?

=> s human and l1  
L2 99 HUMAN AND L1

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=> s clon? or express? or recombinant  
L3 7258991 CLON? OR EXPRESS? OR RECOMBINANT

=> s 2 and l3  
<-----User Break----->

=> s l2 and l3  
L4 49 L2 AND L3

=> dup rem l4  
PROCESSING COMPLETED FOR L4  
L5 22 DUP REM L4 (27 DUPLICATES REMOVED)

=> d 1-22 ibib ab

L5 ANSWER 1 OF 22 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN  
DUPLICATE 1

ACCESSION NUMBER: 2005-11094 BIOTECHDS

TITLE: New interfering RNA that inhibits the **expression** of  
GP131, useful for inducing apoptosis and for treating cancer;  
protein **expression** inhibition using RNA  
interference for use in disease therapy and gene therapy

AUTHOR: OHAGAN R C; KANNAN K; BAILEY D; WRIGHT K; AMARAL L

PATENT ASSIGNEE: GENPATH PHARM INC

PATENT INFO: WO 2005021754 10 Mar 2005

APPLICATION INFO: WO 2004-US27967 27 Aug 2004

PRIORITY INFO: US 2003-498391 27 Aug 2003; US 2003-498391 27 Aug 2003

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2005-202744 [21]

AB DERWENT ABSTRACT:

NOVELTY - An interfering RNA that inhibits the **expression** of  
GP131, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1)  
an antibody that specifically binds to GP131 and inhibits GP131 activity;  
(2) a method of inducing apoptosis in a cell; (3) a method of treating a  
hyperproliferative condition in a mammal; (4) a host cell comprising a  
**recombinant** DNA comprising a GP131-encoding sequence operably  
linked to an **expression** control sequence, where the host cell  
further comprises a genetic mutation that causes the host cell to have a  
greater likelihood of becoming a cancer cell than a cell not comprising  
the genetic mutation; (5) a genetically modified non-human  
mammal at least some of whose cells comprise a genome comprising: (i) a

recombinant GP131-encoding nucleic acid operably linked to an expression control sequence, and (ii) a genetic mutation that causes the mammal to have a greater susceptibility to cancer than a mammal not comprising the genetic mutation, where the genetic modification reduces or eliminates expression of the mammal's endogenous GP131 genes; (6) a screening method for identifying a compound useful for treating a hyperproliferative condition; and (7) a screening method for identifying a compound that inhibits GP115 activity.

BIOTECHNOLOGY - Preferred Interfering RNA: The interfering RNA targets the following sequences: 5'-gatcatcgccgttgaggaa-3', 5'-caaaggcaagcggatatatg-3', or 5'-ctgacatcatcggttactga-3' (SEQ ID NOS: 3-5).

Preferred Genetically Modified Nonhuman Mammal: The genetic mutation is in a tumor suppressor gene. The mammal is a transgenic mammal, all of whose cells comprise a recombinant GP131-encoding nucleic acid operably linked to an expression control sequence, and a genetic mutation that causes the mammal to have a greater susceptibility to cancer than a mammal not comprising the genetic mutation. The mammal is a chimeric mammal at least some of whose, but not all of whose, somatic cells comprise a recombinant

GP131-encoding nucleic acid operably linked to an expression control sequence, and a genetic mutation that causes the mammal to have a greater susceptibility to cancer than a mammal not comprising the genetic mutation, where the percentage of somatic cells comprising a recombinant GP131-encoding nucleic acid operably linked to an expression control sequence, and a genetic mutation that causes the mammal to have a greater susceptibility to cancer is 5-95%, preferably 15-85%. The GP131-encoding nucleic acid is operably linked to a tissue-specific expression system. The genetic modification is a knockout of at least one of the mammal's endogenous GP131 alleles.

The genetic modification is addition of an RNAi expression construct targeting GP131 gene expression. The genetic modification eliminates expression of the mammal's endogenous GP131 genes in a tissue-specific manner. The mammal is chimeric with respect to the genetic modification. Preferred Method: Inducing apoptosis in a cell comprises contacting the cell with an amount of the interfering RNA or contacting the cell with an amount of the antibody. Treating a hyperproliferative condition in a mammal comprises administering to the mammal an amount of the interfering RNA or of the antibody. The method further comprises administering a second therapeutic agent to the mammal, where the second therapeutic agent is an anti-angiogenic agent, anti-metastatic agent, agent that induces hypoxia, agent that induces apoptosis, or an agent that inhibits cell survival signals. Screening method for identifying a compound useful for treating a hyperproliferative condition comprises identifying a biomarker whose level correlates with inhibition of GP131 activity, and detecting a change in the level of the biomarker in the presence of a test compound relative to the level of the biomarker detected in the absence of the test compound. Alternatively, the method comprises providing an inhibitor of GP131 expression or activity, identifying a negative control biomarker pattern formed by biomarkers in a cancer cell, where the cell is not contacted with the inhibitor of GP131 expression or activity, identifying a positive control biomarker pattern formed by biomarkers in the cancer cell, where the cancer cell is contacted with the inhibitor of GP131 expression or activity, identifying a test biomarker pattern formed by biomarkers in the cancer cell, where the cancer cell is contacted with a candidate compound but not contracted with the inhibitor of GP131 expression or activity, and comparing the negative control biomarker pattern, positive control biomarker pattern and test biomarker pattern, detecting a greater similarity between the positive control biomarker pattern and the test biomarker pattern than between the negative control biomarker pattern and the test biomarker pattern. A screening method for identifying a compound that inhibits GP115 activity comprises providing a ceramide preparation containing ceramide and ATP, adding to the preparation a test compound, adding to the preparation an amount of GP131 (ceramide kinase), and detecting a decrease in ceramide phosphate production in the presence of the test compound relative to ceramide phosphate production in the absence of the test compound.

ACTIVITY - Cytostatic. No biological data given.

MECHANISM OF ACTION - Gene Therapy; Apoptosis stimulator; GP131  
ceramide kinase inhibitor.

USE - The interfering RNA, antibody, and method are useful for inducing apoptosis and for treating a hyperproliferative condition, i.e. cancer, e.g. cancers found in skin, lung, prostate, breast, colorectal, liver, pancreatic, brain, testicular, ovarian, uterine, cervical, kidney, thyroid, bladder, esophageal, or hematological tissues. The interfering RNA or antibody is useful for inhibiting tumorigenesis, tumor development, tumor maintenance, tumor recurrence, tumor growth, or growth of tumor cells in vitro.

ADMINISTRATION - Dosage is 0.01-30 mg/kg by oral, injection, topical, transdermal, parenteral, subcutaneous, intrapulmonary, transmucosal, intraperitoneal, intrauterine, sublingual, intrathecal, or intramuscular routes.

EXAMPLE - No relevant example given. (62 pages)

L5 ANSWER 2 OF 22 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on  
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ACCESSION NUMBER: 2005:852065 SCISEARCH

THE GENUINE ARTICLE: 954VJ

TITLE: Basal and induced sphingosine kinase 1 activity in A549 carcinoma cells: function in cell survival and IL-1 beta and TNF-alpha induced production of inflammatory mediators  
AUTHOR: Billich A (Reprint); Bornancin F; Mechtcheriakova D; Natt F; Huesken D; Baumruker T

CORPORATE SOURCE: Novartis Inst BioMed Res, Brunnerstr 59, A-1235 Vienna, Austria (Reprint); Novartis Inst BioMed Res, A-1235 Vienna, Austria; Novartis Inst BioMed Res, CH-4056 Basel, Switzerland  
andreas.billich@phanna.novartis.com

COUNTRY OF AUTHOR: Austria; Switzerland

SOURCE: CELLULAR SIGNALLING, (OCT 2005) Vol. 17, No. 10, pp. 1203-1217.  
ISSN: 0898-6568.

PUBLISHER: ELSEVIER SCIENCE INC, 360 PARK AVE SOUTH, NEW YORK, NY 10010-1710 USA.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 55

ENTRY DATE: Entered STN: 1 Sep 2005

Last Updated on STN: 1 Sep 2005

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Sphingosine-1-phosphate, a lipid mediator produced by sphingosine kinases, regulates diverse cellular processes, ranging from cell growth and survival to effector functions, such as proinflammatory mediator synthesis. Using human A549 epithelial lung carcinoma cells as a model system, we observed transient upregulation of sphingosine kinase type 1 (SPHK1) enzyme activity upon stimulation with both TNF-alpha or IL-1 beta. This transient activation of SPHK1 was found to be required for cytokine-induced COX-2 transcription and PGE(2) production, since not only specific siRNA (abolishing both basal and induced SPHK1 enzyme activity), but also a dominant-negative SPHK1 mutant (suppressing induced SPHK1 activity only) both reduced COX-2 and PGE2. Furthermore, TNF-alpha or IL-1 beta-induced transcription of selected cytokines, chemokines, and adhesion molecules (IL-6, RANTES, MCP-1, and VCAM-1) was found to require SPHK1 activation. Suppression of SPHK1 activation led to reduction of cytokine-induced I kappa B alpha phosphorylation and consequently diminished NF kappa B activity due to reduced nuclear translocation of RelA (p65), explaining the dependence of inflammatory mediator production on SPHK1 activation. Inhibition of basal SPHK1 activity by N,N-dimethylsphingosine or by downregulation of its expression using siRNA induced spontaneous apoptosis in A549 cells, an effect that can be explained through interference with constitutive NF kappa B activity in this cell type. In contrast, expression of the dominant-negative mutant did not induce apoptosis. Taken together, these findings demonstrate a role of SPHK1 activation in proinflammatory signalling and of SPHK1 basal activity in survival of A549 lung carcinoma cells. (c) 2004 Elsevier Inc. All rights reserved.

L5 ANSWER 3 OF 22 MEDLINE on STN DUPLICATE 2  
 ACCESSION NUMBER: 2005367212 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 15900018  
 TITLE: The coordination of prostaglandin E2 production by sphingosine-1-phosphate and ceramide-1-phosphate.  
 AUTHOR: Pettus Benjamin J; Kitatani Kazuyuki; Chalfant Charles E; Taha Tarek A; Kawamori Toshihiko; Bielawski Jacek; Obeid Lina M; Hannun Yusuf A  
 CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, Room 501, Basic Science Building, Medical University of South Carolina, 173 Ashley Avenue, P.O. Box 250509, Charleston, SC 29425, USA.. hannun@musc.edu  
 CONTRACT NUMBER: CA87584 (NCI)  
 GM08716 (NIGMS)  
 GM62887 (NIGMS)  
 HL072925 (NHLBI)  
 SOURCE: Molecular pharmacology, (2005 Aug) 68 (2) 330-5.  
 Electronic Publication: 2005-05-17.  
 Journal code: 0035623. ISSN: 0026-895X.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200508  
 ENTRY DATE: Entered STN: 20050719  
 Last Updated on STN: 20050824  
 Entered Medline: 20050823

AB The ability of pro-inflammatory cytokines such as interleukin-1beta (IL-1beta) to induce the major inflammatory mediator prostaglandin (PG) E(2) depends on the activation of two rate-limiting enzymes, phospholipase A(2) (PLA(2)) and cyclooxygenase 2 (COX-2). PLA(2) acts to generate arachidonic acid, which serves as the precursor substrate for COX-2 in the metabolic pathway leading to PGE(2) production. However, less is known about the mechanisms that coordinate the regulation of these two enzymes. We have provided prior evidence that sphingosine kinase 1 and its bioactive lipid product sphingosine-1-phosphate (S1P) mediate the effects of cytokines on COX-2 induction, whereas ceramide kinase and its distinct product, ceramide-1-phosphate (C1P), are required for the activation and translocation of cPLA(2) (FASEB J 17:1411-1421. 2003; J Biol Chem 278:38206-38213, 2003; J Biol Chem 279:11320-11326, 2004). Herein, we show that these two pathways are independent but coordinated, resulting in synergistic induction of PGE(2). Moreover, the combination of both S1P and C1P recapitulates the temporal and spatial activation of cPLA(2) and with COX-2 seen IL-1beta. Taken together, the results provide, for the first time, a mechanism that assures the coordinate expression and activation in time and space of COX-2 and cPLA(2), assuring maximal production of PGE(2).

L5 ANSWER 4 OF 22 MEDLINE on STN DUPLICATE 3  
 ACCESSION NUMBER: 2005078679 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 15708351  
 TITLE: Characterization of a ceramide kinase-like protein.  
 AUTHOR: Bornancin Frederic; Mechtcheriakova Diana; Stora Samantha; Graf Christine; Wlachos Alexander; Devay Pirooska; Urtz Nicole; Baumruker Thomas; Billich Andreas  
 CORPORATE SOURCE: Novartis Institutes for BioMedical Research, Brunnerstrasse 59, A-1235 Vienna, Austria.. frederic.bornancin@pharma.novartis.com  
 SOURCE: Biochimica et biophysica acta, (2005 Feb 21) 1687 (1-3) 31-43.  
 Journal code: 0217513. ISSN: 0006-3002.  
 PUB. COUNTRY: Netherlands  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200504  
 ENTRY DATE: Entered STN: 20050216  
 Last Updated on STN: 20050419

Entered Medline: 20050418

AB Ceramide is a key player governing cell fate, and its conversion to ceramide-1-phosphate by **ceramide kinase** (CERK) is emerging as an important mean to regulate apoptosis and inflammatory processes. We identified a new **ceramide kinase** homolog, designated CERK-like protein (CERKL) and we compared it to the known CERK. Real time-PCR analysis of human tissues revealed a restricted pattern of **expression** for CERKL mRNA. Surprisingly, various ceramides, known substrates for CERK, were not phosphorylated by CERKL in vitro. Upon 32P(i)-pulse labeling of COS-1 cells transiently **expressing** CERKL, or incubation with NBD-C6-ceramide, ceramide-1-phosphate was not detected. After **recombinant expression** in COS-1 cells, CERKL was partially recovered in the soluble fraction, as a phosphorylated protein. Live cell imaging indicated localization of GFP-tagged CERKL to many cell compartments, including specific association with nucleoli. Two splice variants of CERKL did not localize to nucleoli nor did a CERKL variant with a point mutation in the putative ATP binding site. We also studied a naturally occurring CERKL mutant (R257X), recently linked to the pathology of retinitis pigmentosa. It accumulated in the nucleus but was not associated with nucleoli. Treatment with the calcium ionophore A23187 led to clearing of CERKL from nucleoli, but had no effect on the R257X CERKL mutant. Collectively, although kinase activity of CERKL remains to be proven, these findings suggest a functional link between CERKL and its nucleolar localization. Furthermore, we propose that the cause for retinitis pigmentosa in patients bearing the CERKL R257X mutation might be the accumulation of a truncated CERKL protein in the nucleus.

L5 ANSWER 5 OF 22 MEDLINE on STN  
ACCESSION NUMBER: 2004441014 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 15347320  
TITLE: **Expression of ceramide kinase**  
in hematopoietic cells.  
AUTHOR: Yokota Hiromitsu; Yatomi Yutaka; Mashige Fumiko; Nakahara Kazuhiko  
SOURCE: European journal of haematology, (2004 Oct) 73 (4) 307-8.  
Journal code: 8703985. ISSN: 0902-4441.  
PUB. COUNTRY: Denmark  
DOCUMENT TYPE: Letter  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200412  
ENTRY DATE: Entered STN: 20040908  
Last Updated on STN: 20041219  
Entered Medline: 20041208

L5 ANSWER 6 OF 22 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN  
DUPLICATE 4  
ACCESSION NUMBER: 2004-21354 BIOTECHDS  
TITLE: Novel DNA encoding lipid kinase, useful for treating neurogenic disease, kidney disease, inflammation, congenital immunological syndrome, type II diabetes, obesity, sepsis, arteriosclerosis, cancer or cancer metastasis; involving vector-mediated gene transfer and **expression** in host cell for use in therapy  
PATENT ASSIGNEE: SANKYO CO LTD  
PATENT INFO: JP 2004201681 22 Jul 2004  
APPLICATION INFO: JP 2003-412639 11 Dec 2003  
PRIORITY INFO: JP 2002-359376 11 Dec 2002; JP 2002-359376 11 Dec 2002  
DOCUMENT TYPE: Patent  
LANGUAGE: Japanese  
OTHER SOURCE: WPI: 2004-584000 [57]

AB DERWENT ABSTRACT:  
NOVELTY - A DNA (I) encoding lipid kinase, consisting of or containing 1-1596 nucleotides of sequence (S1) of 1774 nucleotides fully defined in specification, encoding protein having sequence (S2) of 532 amino acids fully defined in specification, is new.  
DETAILED DESCRIPTION - A DNA (I) encoding lipid kinase, consisting of or containing 1-1596 nucleotides of a sequence (S1) of 1774

nucleotides fully defined in the specification, encoding a protein having a sequence (S2) of 532 amino acids fully defined in the specification, hybridizes under stringent conditions with single-stranded DNA having the nucleotide sequence complementary to 1-1596 nucleotides of (S1), contains a region encoding a protein having lipid kinase activity, and shows 95% homology to (S1). INDEPENDENT CLAIMS are also included for: (1) DNA (II) that is inserted into the plasmid comprised in a transformed *Escherichia coli* such as *E. coli* pcDNA3.1-cerk2 SANK 71102 (FERM BP-8201), and a DNA hybridizing under stringent conditions to the above DNA, encodes protein having lipid kinase activity; (2) protein (III) having lipid kinase activity, consisting of or containing (S2), in which one or more amino acids are added, inserted, deleted or substituted, and encoded by (I); (3) protein (IV) comprising the amino acid sequence encoded by (II); (4) recombinant plasmid (V) comprising (I) and (II); (5) a host cell (HC) transformed with (V); (6) an antibody (Ab) specifically coupled with (III) and (IV); (7) preparing (M1) (III) and (IV), involves culturing HC under conditions suitable for producing (III) and (IV) and recovering (III) and (IV); (8) protein obtained by (M1); (9) purifying (M2) (III) and (IV), involves dissolving the sample in a solvent without calmodulin, mixing calmodulin and the resin in a column, washing the resin with a solvent without calmodulin, and eluting (III) and (IV) from the resin by washing with solvent containing ethylenedis(oxyethylenenitrilo)tetraacetic acid (EGTA) or EDTA; (10) detecting (III) and (IV), involves mixing Ab and sample, and detecting Ab and the composite of (III) and (IV), or by mixing sample, lipid substrate and A type phosphoric acid feed stock, and measuring the grade of phosphorylation of lipid substrate; (11) detecting mRNA encoding (III) and (IV) is a sample, involves extracting mRNA from a sample and measuring the amount of mRNA; (12) measuring (M3) lipid kinase activity, involves mixing the sample having one or more lipid substrate, A type phosphoric acid feed stock, (III) and (IV), and cell expressing (III) and (IV), crushing the cell to obtain the cell contents, and detecting the grade of phosphorylation of lipid substrate; (13) screening (M4) substance that controls lipid kinase activity; (14) screening the substance for treating or preventing neurogenic disease, kidney disease, inflammation, congenital immunological syndrome, type II diabetes, obesity, sepsis or arteriosclerosis, by performing the steps of (M4); (15) screening (M5) the substance for treating cancer or cancer metastasis, by performing the steps of (M4); (16) detecting the substance phosphorylated by lipid kinase in a sample, involves mixing (III) and (IV), labeled ceramide, and A-type phosphoric acid feed stock, in the presence and absence of a test sample, and detecting the grade of phosphorylation of a ceramide and selecting the substance based on the grade of phosphorylation; (17) pharmaceutical composition (PC) comprising (III) and (IV), Ab, single or double stranded DNA of (I), single-stranded DNA, RNA or short interfering (si)RNA, and antisense sequence of (I) having 10-50 nucleotides, as an active ingredient; (18) kit for detecting lipid kinase, comprising Ab, primer for PCR amplification of (I) and (II), and a probe that hybridizes under stringent conditions to DNA consisting of or containing (S1) and single-stranded DNA having 10-50 nucleotides, DNA having complementary sequence to (S1) and single-stranded DNA having 20-1000 nucleotides; (19) detecting the risk of a patient suffering from neurogenic disease, kidney disease, inflammation, congenital immunological syndrome, type II diabetes, obesity, sepsis, arteriosclerosis, or cancer and cancer metastasis; (20) screening (M6) the substance that controls the expression of (III) or (IV); (21) screening the substance for treating or preventing neurogenic disease, kidney disease, inflammation, congenital immunological syndrome, type II diabetes, obesity, sepsis, arteriosclerosis, or cancer and cancer metastasis; and (22) screening (M7) the substance that modulates mRNA expression of (III) or (IV);

**BIOTECHNOLOGY** - Preferred DNA: In (I), the lipid kinase phosphorylates the hydroxyl group of the first position of ceramide. Preferred Plasmid: (V) is an expression vector. (V) comprises *E. coli* pcDNA3.1-cerk2 SANK 71102 (FERM BP-8201). Preferred Host Cell: HC is a prokaryotic or eukaryotic cell. HC is derived from mammal. Preferred Antibody: Ab is a polyclonal antibody, monoclonal antibody, humanized antibody, chimeric antibody or human antibody. Preferred Method: In (M5), the lipid substrate is labeled. A-type phosphoric acid



feed stock is 32P-gamma-ATP.

ACTIVITY - Neuroprotective; Nephrotropic; Antiinflammatory; Antidiabetic; Anorectic; Antibacterial; Immunosuppressive; Antiarteriosclerotic; Cytostatic. No supporting data is given.

MECHANISM OF ACTION - Inhibitor of lipid kinase; Vaccine.

USE - (I), (III) and PC is useful for treating neurogenic disease, kidney disease, inflammation, congenital immunological syndrome, type II diabetes, obesity, sepsis, arteriosclerosis, cancer or cancer metastasis (claimed).

ADMINISTRATION - Ab is administered orally, intramuscularly or intravenously, at a dose of 0.1-100 mg/kg, once in 30 days.

EXAMPLE - Cloning of ceramide kinase

cDNA was done as follows. The Marathon-Ready human fetal kidney or fetal lung cDNA (5 microl), oligonucleotide primer (C2-S and C2-A) (0.4 microM), dATP, dGTP, dCTP and dTTP (each 400 microM), magnesium chloride (2.5 mM), LA PCR buffer (50 microl) and LA Taq DNA polymerase (0.05 unit) was prepared, and PCR was carried out. The obtained DNA band was analyzed by 1% agarose gel electrophoresis, and about 700 base pair DNA was observed. The DNA fragment was ligated to pCR2.1 vector and transduced to Escherichia coli INV strain. Plasmid DNA pCR2.1-CERK2S was extracted from the obtained transformant. The fully length DNA sequence was determined by dideoxy nucleotide chain ending method. Results showed that the cDNA had 1774 nucleotides (S1) fully defined in the specification and an open reading frame had the nucleotides 1-1596 of (S1), and protein encoded by DNA comprising (S1), had 532 amino acids. (45 pages)

L5 ANSWER 7 OF 22 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STM  
DUPLICATE 5

ACCESSION NUMBER: 2004-15916 BIOTECHDS

TITLE: Novel ceramide kinase gene promoter DNA,  
useful for screening substance promoting or suppressing  
expression level of ceramide kinase

new promoter for use in gene expression level  
control and expression of luciferase,  
peroxidase, alkaline phosphatase, beta-galactosidase or  
green fluorescent protein in a mammal cell

PATENT ASSIGNEE: SANKYO CO LTD

PATENT INFO: JP 2004081147 18 Mar 2004

APPLICATION INFO: JP 2002-249220 28 Aug 2002

PRIORITY INFO: JP 2002-249220 28 Aug 2002; JP 2002-249220 28 Aug 2002

DOCUMENT TYPE: Patent

LANGUAGE: Japanese

OTHER SOURCE: WPI: 2004-320965 [30]

AB DERWENT ABSTRACT:

NOVELTY - A DNA having promoter activity in mammalian cells, chosen from a DNA containing a nucleotide sequence having a fully defined sequence of 2515 base pairs (S1) as given in the specification, a DNA having a nucleotide sequence which hybridizes under stringent conditions with DNA which consists of a nucleotide sequence complementary to (S1), or a DNA having nucleotide sequence exhibiting 95% or more sequence identity with (S1), is new.

DETAILED DESCRIPTION - A DNA (I) having promoter activity in mammalian cells, chosen from: (a) a DNA containing a nucleotide sequence having a fully defined sequence of 2515 base pairs (S1) as given in the specification, or a DNA having (S1) in which nucleotides at position 1087 act as 5' terminal and the nucleotides at position 2515 act as 3' terminal; (b) a DNA having a nucleotide sequence which hybridizes under stringent conditions with DNA which consist of a nucleotide sequence complementary to (S1); (c) a DNA having nucleotide sequence exhibiting 95% or more sequence identity with (S1); (d) a DNA having nucleotide sequences from the position 1087-2515 of (S1); or (e) a DNA containing sequences from position 1087-2515 of (S1) whose 5' terminal contains a 1086 base pair polynucleotide or a nucleotide which is one or more base pair longer than 1086 base pair polynucleotide. INDEPENDENT CLAIMS are included for the following (1) a recombinant plasmid (II) containing (I), maintained in a transformed Escherichia coli pGV-hcerkp2 SANK 70802 (FERM BP-8087), where (I) is connected to 5' terminal of a

nucleotide sequence which encodes a firefly luciferase and/or the 3' terminal of (I) is connected to a DNA which consists of 1429 base pairs; (2) a host cell (III) in which (II) is transduced; (3) transforming E.coli pGV-hcerkp2 SANK 70802 (FERM BP-8087); (4) identifying (M1) cDNA encoding a protein which treats or prevents diseases such as neurogenic disease, inflammation, infectious disease, thrombosis, immunological disease, HIV, type II diabetes, obesity, sepsis, cardiac disease, abnormality in a lipid metabolism, or arteriosclerosis, involves: (a) cultivating a cell (A) in which (II) is introduced, a cell (B) in which a mammalian **expression** vector comprising a test cDNA which is transiently or stably **expressed**, and a cell (C) containing a **recombinant** plasmid which does not contain a test cDNA; (b) measuring the **expression** level of the protein encoded by the DNA which is connected to 3' terminal of (I); (c) comparing the **expression** level of the protein in cell (A), (B) and (C); and (d) identifying the protein having therapeutic or preventive effect, based on the increased level of **expression** of the protein in cell (B) than in cell (A) or (C); (5) identifying cDNA encoding a protein which treats or prevents cancer or metastasis, involves carrying out the above mentioned steps of (M1), and identifying the protein having therapeutic or preventive effect, based on the decreased level of **expression** of the protein in cell (B) than in cell (A) or (C); (6) selecting (M2) a substance which controls **ceramide kinase** gene promoter activity, involves: (a) adding nucleus extract of human cell and to-be-tested substance, and nucleus extract of human cell, to the labeled DNA; (b) electrophoresing the prepared mixture; (c) detecting the band which contains labeled DNA; (d) comparing mobility between the band obtained from the sample containing nucleus extract and the band obtained from the sample containing both the nucleus extract and to-be-tested substance; and (e) selecting the substance which exhibited increased mobility; and (7) a nucleotide sequence or its derivative controlling **ceramide kinase** gene promoter activity, comprising 10 or more nucleotides of (S1), or complementary nucleotide sequences to the above mentioned nucleotides.

BIOTECHNOLOGY - Preferred Recombinant Plasmid: In (II), a DNA encoding a protein is connected to 3' terminal of (I), where the protein is chosen from luciferase, Western horseradish peroxidase, alkaline phosphatase, beta-galactosidase and green fluorescent protein, or preferably luciferase. Preferred Host Cell: (III) is of mammalian origin. Preferred Method: In (M2), the DNA is radiolabeled.

ACTIVITY - Neuroprotective; Antiinflammatory; Thrombolytic; Immunomodulatory; Anti-HIV; Antidiabetic; Anorectic; Antibacterial; Immunosuppressive; Cardiant; Antiarteriosclerotic; Cytostatic. No suitable biological data given.

MECHANISM OF ACTION - None given.

USE - (I) is useful for isolating a substance which controls **ceramide kinase** gene promoter activity, which involves contacting a sample containing the substance with (I), and isolating the substance that is bound to (I). (I) is useful for detecting modulator of **ceramide kinase** gene promoter activity, which involves contacting a sample containing nucleus extract of human cell with (I), and isolating a protein bound to (I). (III) is useful for screening a substance which regulates **ceramide kinase** gene promoter activity, which involves cultivating (III) in the presence or absence of a to-be-tested substance, detecting the **expression** level of the protein encoded by DNA connected to 3' terminal of (I), comparing the **expression** level of protein between the cell culture cultivated in the presence and absence of the to-be-tested substance. (III) is useful for screening a substance which prevents or treats diseases such as neurogenic disease, inflammation, infectious disease, thrombosis, immunological disease, HIV, type II diabetes, obesity, sepsis, cardiac disease, abnormality in a lipid metabolism, or arteriosclerosis, which involves carrying out the above mentioned steps, and identifying the to-be-tested substance having treatment or preventive effect, based on the increased level of the **expression** of the protein. (III) is also useful for screening a substance which treats or prevents metastasis or cancer, which involves carrying out the above mentioned steps, and identifying the to-be-tested substance having treatment or preventive effect, based on the decreased level of the

**expression** of the protein. (III) is useful for identifying cDNA encoding a protein which treats or prevents the above mentioned diseases (claimed).

ADVANTAGE - (I) enables screening of a substance that promotes or suppresses the expression level of ceramide kinase.

EXAMPLE - DNA containing a fully defined sequence of 2515 base pairs as given in the specification was chosen from database. The sequences at positions 1286-1535 were identified as promoter region. To the promoter DNA, at the 5' terminal, a nucleotide encoding luciferase was introduced. The obtained promoter DNA was then introduced into a plasmid vector to prepare recombinant plasmids such as pGV-hcerkp1 and pGV-hcerkp2. Human genomic DNA was taken as a template and polymerase chain reaction (PCR) was performed using primers having sequences such as 5'-tccagtgtctgtgccagagtcattgg-3', 5'-atctccgccgccgggtctgtccgc-3' and 5'-atccacatttcccaggcctcagagc-3'. The PCR product was recovered and inserted into pCR2.1 vector. The vector was then introduced into Escherichia coli INV(alpha)F' strain. The nucleotide sequences of the inserted DNA was analyzed. The plasmid which contained 2515 base pair DNA and a plasmid which contained 1087-2515 base pairs of the 2515 base pair DNA were referred as pCR2.1-hcerkp1 and pCR2.1-hcerkp2, respectively. A 2.5 kbp fragment of pCR2.1-hcerkp2 obtained by restriction enzyme digestion was ligated with pGV-B2 and introduced into E.coli JM109 strain. A 1.5 kbp fragment of pCR2.1-hcerkp1 obtained by restriction enzyme digestion was ligated with pGV-B2. The plasmids such as pGV-B2, pGV-hcerkp1 and pGV-hcerkp2 were introduced into HEK293 cell. The cells were cultivated at 37 degrees C for 2 days and the luciferase activity was measured. The plasmid pGV-hcerkp1 and pGV-hcerkp2 exhibited the luciferase activity which was 27 and 24 times greater than that of pGV-B2. (24 pages)

L5 ANSWER 8 OF 22 HCAPLUS COPYRIGHT 2005 ACS on STN  
ACCESSION NUMBER: 2004:1059473 HCAPLUS  
DOCUMENT NUMBER: 142:34471  
TITLE: Cloning, sequences, and drug screening and  
diagnostic use of human ceramide  
kinase-like proteins  
INVENTOR(S): Bornancin, Frederic  
PATENT ASSIGNEE(S): Novartis A.-G., Switz.; Novartis Pharma G.m.b.H.  
SOURCE: PCT Int. Appl., 40 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004106509	A1	20041209	WO 2004-EP3740	20040407
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW			
RW:	BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG			

PRIORITY APPLN. INFO.: US 2003-474239P P 20030529

AB The nucleotide sequences and the encoded amino acid sequences of human ceramide kinase-like proteins are provided. The ceramide kinase-like proteins of the invention can be used in drug screening and diagnostics.

REFERENCE COUNT: 1 THERE ARE 1 CITED REFERENCES AVAILABLE FOR THIS  
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 9 OF 22 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2004:802537 HCAPLUS  
 DOCUMENT NUMBER: 141:289087  
 TITLE: **Expression** and screening for compounds  
 regulating activity of **ceramide**  
**kinase** in tissues, for use in treatment of  
**human** diseases  
 INVENTOR(S): Kossida, Sophia; Encinas, Jeffrey; Takao, Eiko  
 PATENT ASSIGNEE(S): Bayer Aktiengesellschaft, Germany  
 SOURCE: U.S. Pat. Appl. Publ., 50 pp., Cont.-in-part of U.S.  
 Ser. No. 969,896, abandoned.  
 CODEN: USXXCO  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY ACC. NUM. COUNT: 2  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2004192580	A1	20040930	US 2003-631958	20031219
US 2003125533	A1	20030703	US 2001-969896	20011004
PRIORITY APPLN. INFO.:			US 2000-238005P	P 20001006
			US 2001-314113P	P 20010823
			US 2001-969896	B2 20011004

AB This invention relates to **expression** and screening for compds.  
 regulating activity of **ceramide kinase** in tissues, for  
 use in treatment of **human** diseases. **Ceramide**  
**kinase** cDNA and protein sequences, as well as **expression**  
 profiles in various **human** tissues and cell lines, are provided.  
 Reagents that regulate **human ceramide kinase**  
 protein activity and reagents that bind to **human**  
**ceramide kinase** gene products can be used to regulate  
 intracellular signaling and consequently cell proliferation and apoptosis.  
 Methods of drug screening for reagents influencing **ceramide**  
**kinase** activity in HEK293 cells was exemplified by use of  
 sphingosine derivs., in conjunction with anal. of cellular apoptotic  
 response. Such regulation is particularly useful for treating allergies  
 including but not limited to asthma, autoimmune diseases such as  
 rheumatoid arthritis, inflammatory disease, transplant rejection, and  
 cancer, particularly lymphocytic leukemias, and could be a useful target  
 of vaccination enhancing adjuvants. Central and peripheral nervous system  
 disorders, such as Parkinson's disease, also can be treated.

L5 ANSWER 10 OF 22 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on  
 STN

ACCESSION NUMBER: 2004:802806 SCISEARCH  
 THE GENUINE ARTICLE: 851KN  
 TITLE: MuLK, a eukaryotic multi-substrate lipid kinase  
 AUTHOR: Waggoner D W; Johnson L B; Mann P C; Morris V; Guastella  
 J; Bajjalieh S M (Reprint)  
 CORPORATE SOURCE: Univ Washington, Dept Pharmacol, Box 357280, Seattle, WA  
 98195 USA (Reprint); Univ Washington, Dept Pharmacol,  
 Seattle, WA 98195 USA  
 bajjalie@u.washington.edu  
 COUNTRY OF AUTHOR: USA  
 SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (10 SEP 2004) Vol. 279,  
 No. 37, pp. 38228-38235.  
 ISSN: 0021-9258.  
 PUBLISHER: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650  
 ROCKVILLE PIKE, BETHESDA, MD 20814-3996 USA.  
 DOCUMENT TYPE: Article; Journal  
 LANGUAGE: English  
 REFERENCE COUNT: 39  
 ENTRY DATE: Entered STN: 2 Oct 2004  
 Last Updated on STN: 2 Oct 2004

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB We report the identification and characterization of a novel lipid  
 kinase that phosphorylates multiple substrates. This enzyme, which we  
 term MuLK for multisubstrate lipid kinase, does not belong to a previously  
 described lipid kinase family. MuLK has orthologs in many organisms and

is broadly **expressed** in human tissues. Although predicted to be a soluble protein, MuLK co-fractionates with membranes and localizes to an internal membrane compartment. **Recombinant** MuLK phosphorylates diacylglycerol, ceramide, and 1-acylglycerol but not sphingosine. Although its affinity for diacylglycerol and ceramide are similar, MuLK exhibits a higher V-max toward diacylglycerol in vitro, consistent with it acting primarily as a diacylglycerol kinase. MuLK activity was inhibited by sphingosine and enhanced by cardiolipin. It was stimulated by calcium when magnesium concentrations were low and inhibited by calcium when magnesium concentrations were high. The effects of charged lipids and cations on MuLK activity in vitro suggest that its activity in vivo is tightly regulated by cellular conditions.

L5 ANSWER 11 OF 22 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.  
on STN

ACCESSION NUMBER: 2004410847 EMBASE  
TITLE: **Expression of ceramide kinase**  
in hematopoietic cells.  
AUTHOR: Yokota H.; Yatomi Y.; Mashige F.; Nakahara K.  
CORPORATE SOURCE: Dr. Y. Yatomi, Department of Laboratory Medicine, Graduate  
School of Medicine, University of Tokyo, 7-3-1 Hongo,  
Bunkyo-ku, Tokyo 113-8655, Japan. yatomiy-lab@h.u-  
tokyo.ac.jp  
SOURCE: European Journal of Haematology, (2004) Vol. 73, No. 4, pp.  
307-308.  
Refs: 8  
ISSN: 0902-4441 CODEN: EJHAEC  
COUNTRY: United Kingdom  
DOCUMENT TYPE: Journal; Letter  
FILE SEGMENT: 029 Clinical Biochemistry  
LANGUAGE: English  
ENTRY DATE: Entered STN: 20041018  
Last Updated on STN: 20041018

L5 ANSWER 12 OF 22 MEDLINE on STN DUPLICATE 6

ACCESSION NUMBER: 2003608046 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 14681825  
TITLE: **Mutation of CERKL, a novel human ceramide**  
**kinase gene, causes autosomal recessive retinitis**  
**pigmentosa (RP26).**  
AUTHOR: Tuson Miquel; Marfany Gemma; Gonzalez-Duarte Roser  
CORPORATE SOURCE: Departament de Genetica, Facultat de Biologia, Universitat  
de Barcelona, Barcelona, Spain.  
SOURCE: American journal of human genetics, (2004 Jan) 74 (1)  
128-38. Electronic Publication: 2003-12-16.  
Journal code: 0370475. ISSN: 0002-9297.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-AB079066; GENBANK-AB079067; GENBANK-AK112588;  
GENBANK-AK112750; GENBANK-AY128394; GENBANK-AY357073;  
GENBANK-BC020465; GENBANK-BC046474; GENBANK-BE797822;  
GENBANK-BY742285; GENBANK-Q01583; GENBANK-Q09103;  
GENBANK-Q39017  
ENTRY MONTH: 200403  
ENTRY DATE: Entered STN: 20031224  
Last Updated on STN: 20040302  
Entered Medline: 20040301

AB Retinitis pigmentosa (RP), the main cause of adult blindness, is a genetically heterogeneous disorder characterized by progressive loss of photoreceptors through apoptosis. Up to now, 39 genes and loci have been implicated in nonsyndromic RP, yet the genetic bases of >50% of the cases, particularly of the recessive forms, remain unknown. Previous linkage analysis in a Spanish consanguineous family allowed us to define a novel autosomal recessive RP (arRP) locus, RP26, within an 11-cM interval (17.4 Mb) on 2q31.2-q32.3. In the present study, we further refine the RP26 locus down to 2.5 Mb, by microsatellite and single-nucleotide polymorphism (SNP) homozygosity mapping. After unsuccessful mutational analysis of the

nine genes initially reported in this region, a detailed gene search based on **expressed-sequence-tag** data was undertaken. We finally identified a novel gene encoding a **ceramide kinase** (CERKL), which encompassed 13 exons. All of the patients from the RP26 family bear a homozygous mutation in exon 5, which generates a premature termination codon. The same mutation was also characterized in another, unrelated, Spanish pedigree with arRP. Human CERKL is **expressed** in the retina, among other adult and fetal tissues. A more detailed analysis by in situ hybridization on adult murine retina sections shows **expression** of Cerkl in the ganglion cell layer. **Ceramide kinases** convert the sphingolipid metabolite ceramide into ceramide-1-phosphate, both key mediators of cellular apoptosis and survival. Ceramide metabolism plays an essential role in the viability of neuronal cells, the membranes of which are particularly rich in sphingolipids. Therefore, CERKL deficiency could shift the relative levels of the signaling sphingolipid metabolites and increase sensitivity of photoreceptor and other retinal cells to apoptotic stimuli. This is the first genetic report suggesting a direct link between retinal neurodegeneration in RP and sphingolipid-mediated apoptosis.

L5 ANSWER 13 OF 22 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on  
STN DUPLICATE 7

ACCESSION NUMBER: 2005:50436 BIOSIS  
DOCUMENT NUMBER: PREV200500045870  
TITLE: Trophic effects of insulin-like growth factor-I (IGF-I) in the inner ear.  
AUTHOR(S): Varela-Nieto, Isabel [Reprint Author]; Morales-Garcia, Jose A.; Vigil, Patricia; Diaz-Casares, Amelia; Gorospe, Itziar; Sanchez-Galiano, Susana; Canon, Susana; Camarero, Guadalupe; Contreras, Julio; Cediell, Rafael; Leon, Yolanda  
CORPORATE SOURCE: Consejo Super Invest CientificasInst Invest Biomed Alberto Sols, Univ Autonoma Madrid, Arturo Duperier 4, Madrid, 28029, Spain  
ivarela@iib.uam.es  
SOURCE: Hearing Research, (October 2004) Vol. 196, No. 1-2, pp. 19-25. print.  
ISSN: 0378-5955 (ISSN print).  
DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 26 Jan 2005  
Last Updated on STN: 26 Jan 2005

AB Insulin-like growth factors (IGFs) have a pivotal role during nervous system development and in its functional maintenance. IGF-I and its high affinity receptor (IGF1R) are **expressed** in the developing inner ear and in the postnatal cochlear and vestibular ganglia. We recently showed that trophic support by IGF-I is essential for the early neurogenesis of the chick cochleovestibular ganglion (CVG). In the chicken embryo otic vesicle, IGF-I regulates developmental death dynamics by regulating the activity and/or levels of key intracellular molecules, including lipid and protein kinases such as **ceramide kinase**, Akt and Jun N-terminal kinase (JNK). Mice lacking IGF-I lose many auditory neurons and present increased auditory thresholds at early postnatal ages. Neuronal loss associated to IGF-I deficiency is caused by apoptosis of the auditory neurons, which presented abnormally increased levels of activated caspase-3. It is worth noting that in man, homozygous deletion of the IGF-I gene causes sensory-neural deafness (reviewed in Rev. Endo. Met. Disord. 3 (2002) 357). IGF-I is thus necessary for normal development and maintenance of the inner ear. The trophic action of IGF-I in the inner ear suggest that this factor may have therapeutic potential for the treatment of hearing loss. Copyright 2004 Elsevier B.V. All rights reserved.

L5 ANSWER 14 OF 22 MEDLINE on STN DUPLICATE 8

ACCESSION NUMBER: 2003554351 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 13129923  
TITLE: Phosphorylation of the immunomodulatory drug FTY720 by sphingosine kinases.  
AUTHOR: Billich Andreas; Bornancin Frederic; Devay Pirooska; Mechtcheriakova Diana; Urtz Nicole; Baumruker Thomas

CORPORATE SOURCE: Novartis Research Institute, Brunnerstrasse 59, A-1235  
Vienna, Austria.. andreas.billich@pharma.novartis.com  
SOURCE: Journal of biological chemistry, (2003 Nov 28) 278 (48)  
47408-15. Electronic Publication: 2003-09-16.  
Journal code: 2985121R. ISSN: 0021-9258.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200401  
ENTRY DATE: Entered STN: 20031125  
Last Updated on STN: 20040113  
Entered Medline: 20040112

AB The immunomodulatory drug FTY720 is phosphorylated in vivo, and the resulting FTY720 phosphate as a ligand for sphingosine-1-phosphate receptors is responsible for the unique biological effects of the compound. So far, phosphorylation of FTY720 by murine sphingosine kinase (SPHK) 1a had been documented. We found that, while FTY720 is also phosphorylated by human SPHK1, the human type 2 isoform phosphorylates the drug 30-fold more efficiently, because of a lower Km of FTY720 for SPHK2. Similarly, murine SPHK2 was more efficient than SPHK1a. Among splice variants of the human SPHKs, an N-terminally extended SPHK2 isoform was even more active than SPHK2 itself. Further SPHK superfamily members, namely ceramide kinase and a "SPHK-like" protein, failed to phosphorylate sphingosine and FTY720. Thus, only SPHK1 and 2 appear to be capable of phosphorylating FTY720. Using selective assay conditions, SPHK1 and 2 activities in murine tissues were measured. While activity of SPHK2 toward sphingosine was generally lower than of SPHK1, FTY720 phosphorylation was higher under conditions favoring SPHK2. In human endothelial cells, while activity of SPHK1 toward sphingosine was 2-fold higher than of SPHK2, FTY720 phosphorylation was 7-fold faster under SPHK2 assay conditions. Finally, FTY720 was poorly phosphorylated in human blood as compared with rodent blood, in line with the low activity of SPHK1 and in particular of SPHK2 in human blood. To conclude, both SPHK1 and 2 are capable of phosphorylating FTY720, but SPHK2 is quantitatively more important than SPHK1.

L5 ANSWER 15 OF 22 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on  
STN

ACCESSION NUMBER: 2004:139915 BIOSIS  
DOCUMENT NUMBER: PREV200400133469  
TITLE: Enhancement in phagocytosis by human  
ceramide kinase transfection of  
FcgammaRIIA bearing COS-1 cells.  
AUTHOR(S): Hinkovska-Galcheva, Vania Tz [Reprint Author]; Hiraoka,  
Miki; Abe, Akira; Boxer, Laurence A.; Shayman, James A.  
CORPORATE SOURCE: Pediatrics/Hematology, University of Michigan, Ann Arbor,  
MI, USA  
SOURCE: Blood, (November 16 2003) Vol. 102, No. 11, pp. 272a.  
print.  
Meeting Info.: 45th Annual Meeting of the American Society  
of Hematology. San Diego, CA, USA. December 06-09, 2003.  
American Society of Hematology.  
CODEN: BLOOAW. ISSN: 0006-4971.  
DOCUMENT TYPE: Conference; (Meeting)  
Conference; (Meeting Poster)  
Conference; Abstract; (Meeting Abstract)  
LANGUAGE: English  
ENTRY DATE: Entered STN: 10 Mar 2004  
Last Updated on STN: 10 Mar 2004

AB The agonist-stimulated metabolism of membrane lipids produces potent second messengers that regulate phagocytosis. We identified the generation of ceramide-1-phosphate (C1P) through activation of a ceramide kinase in neutrophils. Our previous studies indicate that the addition of (C1P), to liposomes enhanced the rate and extent of the calcium-dependent fusion. We now hypothesize that human ceramide kinase (hCERK) activity and C1P

synthesis leads to enhanced phagocytosis. A plasmid carrying hCERK was transiently transfected into COS-1 cells carrying FcgammaRIIA. Our transfection efficiency was 40-50% as measured by co-transfection with a beta-galactosidase expression construct. Ceramide kinase activity was significantly higher in cells transfected with hCERK (29.4+-17 pmol/min/106 cells p<0.04) than in the FcgammaRIIA cells (1.18+-0.9 pmol/min/106 cells) or cells transfected with pc-vector (1.43+-1.35 pmol/min/106). Additionally hCERK transfection resulted in increasing FcgammaRIIA-mediated phagocytosis by 40% without significantly reducing ceramide levels. Besides increasing the phagocytic index, the percentage of ingesting COS-1 cells increased from 43+-11 in the control cells and 50+-11 in the vector control to 70+-9 (p<0.0001, n=6) in hCERK transfected cells. To further examine the role of hCERK activity and C1P synthesis in enhancing phagocytosis we created stable transfectants simultaneously carrying FcgammaRIIA and hCERK. Stably transfected cells showed a 3-fold increase in phagocytic index. Labeling resting cells with (32P)H3PO4 or independently with (3H)-D-erythro-sphingosine revealed increased ceramide-1-phosphate levels. Cells labeled with (3H)-D-erythro-sphingosine and challenged with E1gG showed increased phagocytosis by three fold and simultaneously increased C1P levels by 29% compared to resting controls. In conclusion transfected COS-1 cells were able to increase their C1P levels during phagocytosis. In turn hCERK transfection results in a novel means to markedly enhance phagocytosis.

L5 ANSWER 16 OF 22 MEDLINE on STN DUPLICATE 9  
 ACCESSION NUMBER: 2003390067 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 12927832  
 TITLE: A specific ceramide kinase assay to measure cellular levels of ceramide.  
 AUTHOR: Bektas Meryem; Jolly Puneet S; Milstien Sheldon; Spiegel Sarah  
 CORPORATE SOURCE: Medical College of Virginia Campus, Virginia Commonwealth University, Richmond, VA 23298-0614, USA.  
 CONTRACT NUMBER: CA61774 (NCI)  
 SOURCE: Analytical biochemistry, (2003 Sep 15) 320 (2) 259-65.  
 Journal code: 0370535. ISSN: 0003-2697.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200404  
 ENTRY DATE: Entered STN: 20030821  
 Last Updated on STN: 20040415  
 Entered Medline: 20040414

AB Human ceramide kinase was recently cloned and characterized. Recombinant ceramide kinase is highly active and ceramide is the only lipid that it phosphorylates, indicating that it should be useful for the measurement of ceramide levels in biological samples by conversion to ceramide-1-phosphate, in a manner analogous to that of the widely used Escherichia coli diacylglycerol kinase method. Using recombinant ceramide kinase, we have now developed a rapid and specific enzymatic method to quantify mass levels of long-chain ceramides in cellular lipid extracts. This new ceramide kinase assay is more specific than the commonly used diacylglycerol kinase method because the ubiquitous lipid diacylglycerol, the preferred substrate for diacylglycerol kinase which is usually present at higher concentrations than ceramide and can interfere with ceramide phosphorylation, is completely inactive with ceramide kinase. Moreover, this high specificity eliminates the need for analysis of the lipid product by thin-layer chromatography since ceramide-1-phosphate is the only radiolabeled lipid in organic solvent extracts of ceramide kinase reactions.

L5 ANSWER 17 OF 22 MEDLINE on STN DUPLICATE 10  
 ACCESSION NUMBER: 2003109977 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 12624491  
 TITLE: Ceramide 1-phosphate formation in neutrophils.  
 AUTHOR: Rile Ge; Yatomi Yutaka; Takafuta Toshiro; Ozaki Yukio



CORPORATE SOURCE: Department of Laboratory Medicine, Yamanashi Medical University, Japan.  
 SOURCE: Acta haematologica, (2003) 109 (2) 76-83.  
 Journal code: 0141053. ISSN: 0001-5792.  
 PUB. COUNTRY: Switzerland  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200303  
 ENTRY DATE: Entered STN: 20030308  
 Last Updated on STN: 20030328  
 Entered Medline: 20030327

AB In this study, we examined the metabolism of [(3)H]N-hexanoylsphingosine [C(6)-ceramide (Cer)] in neutrophils, erythrocytes, platelets and mononuclear cells. [(3)H]C(6)-Cer, exogenously added and incorporated into the inside of the cell, was found to be converted to other radioactive sphingolipids in these differentiated blood cells, except erythrocytes, which were unable to metabolize C(6)-Cer. Only in neutrophils was a specific radioactive band, comigrating with a standard C(6)-Cer 1-phosphate (C(6)-Cer-1-P) on thin layer chromatography, observed in a time-dependent manner. This metabolite was confirmed to be C(6)-Cer-1-P by its sensitivity to acid treatment and resistance to mild alkaline hydrolysis. Neutrophil [(3)H]C(6)-Cer conversion into [(3)H]C(6)-Cer-1-P, reflecting Cer kinase activity, was not affected by cell stimulation. Furthermore, extracellular release of [(3)H]C(6)-Cer-1-P was not observed either. Exogenous addition of C(8)-Cer-1-P failed to induce intracellular Ca(2+) mobilization or affect the response induced by the formyl peptide. Furthermore, neutrophil morphology was not affected by C(8)-Cer-1-P. Although specific expression of Cer kinase suggests an important role for Cer-1-P in neutrophils, its functional role(s) remain to be clarified.  
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L5 ANSWER 18 OF 22 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2002:504646 HCAPLUS  
 DOCUMENT NUMBER: 137:83610  
 TITLE: Compositions and methods for the treatment and prevention of cardiovascular diseases and disorders, and for identifying agents therapeutic therefor  
 INVENTOR(S): Sabbadini, Roger A.  
 PATENT ASSIGNEE(S): Medlyte, Inc., USA  
 SOURCE: PCT Int. Appl., 188 pp.  
 CODEN: PIXXD2  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002051439	A2	20020704	WO 2001-US50785	20011221
WO 2002051439	A3	20030814		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
CA 2432978	AA	20020704	CA 2001-2432978	20011221
US 2003026799	A1	20030206	US 2001-28156	20011221
US 6881546	B2	20050419		
US 2003027304	A1	20030206	US 2001-29401	20011221
US 6858383	B2	20050222		
US 2003096022	A1	20030522	US 2001-29372	20011221
EP 1363643	A2	20031126	EP 2001-987517	20011221

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,  
IE, SI, LT, LV, FI, RO, MK, CY, AL, TR

US 2004247603 A1 20041209 US 2004-820582 20040407  
PRIORITY APPLN. INFO.: US 2000-257926P P 20001222  
US 2001-28156 A3 20011221  
WO 2001-US50785 W 20011221

OTHER SOURCE(S): MARPAT 137:83610

AB Methods and compns. are disclosed that are useful for the prevention and/or treatment of cardiovascular and cardiac diseases and disorders, or damage resulting from surgical or medical procedures that may cause ischemic or ischemic/reperfusion damage in humans; and cardiovascular trauma. The beneficial effects of the compns. and methods are achieved through the use of pharmaceutical compns. that include agents that interfere with the production and/or biol. activities of sphingolipids and their metabolites, particularly sphingosine (SPH) and sphingosine-1-phosphate (S-1-P). Also disclosed are methods for identifying and isolating therapeutic agents.

L5 ANSWER 19 OF 22 MEDLINE on STN DUPLICATE 11

ACCESSION NUMBER: 2002347257 MEDLINE

DOCUMENT NUMBER: PubMed ID: 11956206

TITLE: **Ceramide kinase**, a novel lipid kinase.  
Molecular cloning and functional characterization.

AUTHOR: Sugiura Masako; Kono Keita; Liu Hong; Shimizugawa Tetsuya;  
Minekura Hiroyuki; Spiegel Sarah; Kohama Takafumi

CORPORATE SOURCE: Pharmacology and Molecular Biology Research Laboratories,  
Sankyo Co., Ltd., Tokyo 140-8710, Japan.

SOURCE: Journal of biological chemistry, (2002 Jun 28) 277 (26)  
23294-300. Electronic Publication: 2002-04-15.  
Journal code: 2985121R. ISSN: 0021-9258.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

OTHER SOURCE: GENBANK-AB079066; GENBANK-AB079067

ENTRY MONTH: 200208

ENTRY DATE: Entered STN: 20020702

Last Updated on STN: 20030105

Entered Medline: 20020806

AB Ceramide-1-phosphate is a sphingolipid metabolite that has been implicated in membrane fusion of brain synaptic vesicles and neutrophil phagolysosome formation. Ceramide-1-phosphate can be produced by ATP-dependent **ceramide kinase** activity, although little is known of this enzyme because it has not yet been highly purified or **cloned**. Based on sequence homology to sphingosine kinase type 1, we have now **cloned** a related lipid kinase, **human ceramide kinase** (hCERK). hCERK encodes a protein of 537 amino acids that has a catalytic region with a high degree of similarity to the diacylglycerol kinase catalytic domain. hCERK also has a putative N-myristoylation site on its NH(2) terminus followed by a pleckstrin homology domain. Membrane but not cytosolic fractions from HEK293 cells transiently transfected with a hCERK **expression** vector readily phosphorylated ceramide but not sphingosine or other sphingoid bases, diacylglycerol or phosphatidylinositol. This activity was clearly distinguished from those of bacterial or **human** diacylglycerol kinases. With natural ceramide as a substrate, the enzyme had a pH optimum of 6.0-7.5 and showed Michaelis-Menten kinetics, with K(m) values of 187 and 32 microm for ceramide and ATP, respectively. Northern blot analysis revealed that hCERK mRNA **expression** was high in the brain, heart, skeletal muscle, kidney, and liver. A BLAST search analysis using the hCERK sequence revealed that putative **ceramide kinases** (CERKs) exist widely in diverse multicellular organisms including plants, nematodes, insects, and vertebrates. Phylogenetic analysis revealed that CERKs are a new class of lipid kinases that are clearly distinct from sphingosine and diacylglycerol kinases. **Cloning** of CERK should provide new molecular tools to investigate the physiological functions of ceramide-1-phosphate.

L5 ANSWER 20 OF 22 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN  
DUPLICATE 12

ACCESSION NUMBER: 2002-09980 BIOTECHDS

TITLE: Human ceramide kinase gene and  
the enzyme encoded by it for screening substances as drugs  
for neurological, inflammatory and other disorders;  
recombinant enzyme gene, vector  
expression in host cell, antibody, database and  
polymerase chain reaction useful in disease gene therapy

AUTHOR: SUGIURA M; KONO K; KOHAMA T

PATENT ASSIGNEE: SANKYO CO LTD

PATENT INFO: WO 2001096575 20 Dec 2001

APPLICATION INFO: WO 2000-JP4889 14 Jun 2000

PRIORITY INFO: JP 2000-178039 14 Jun 2000

DOCUMENT TYPE: Patent

LANGUAGE: Japanese

OTHER SOURCE: WPI: 2002-179513 [23]

AB DERWENT ABSTRACT:

NOVELTY - A ceramide kinase (CERK1) of human  
origin having a 537 residue amino acid sequence fully defined in the  
specification, also ceramide kinase enzymes derived  
from this sequence by addition, deletion and/or substitution of one or  
more amino acid residues, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the  
following: (1) DNA encoding the novel ceramide kinase  
; (2) expression vectors containing the DNA of (1); (3) host  
cells transformed by the vectors of (2); (4) preparing the enzyme by  
culture of the transformed cells of (3); (5) isolation of the enzyme from  
samples containing it by co-absorption with calmodulin on to an affinity  
column, and elution from the column by a solvent containing  
ethylenedis(oxyethylenetri)l(tetraacetic acid (EGTA) or  
ethylenediaminetetraacetic acid (EDTA); (6) screening compounds for their  
ability to inhibit the ceramide kinase by measuring  
the extent of phosphorylation of a substrate for the enzyme in the  
presence or absence of the inhibitor; (7) kits for the screening method  
of (6); (8) antibodies binding to the enzyme; (9) drug compositions  
containing DNA encoding all or part of the enzyme; (10) oligonucleotides  
15-30 bases in length hybridizing to DNA encoding the enzyme; and (11)  
drug compositions containing the oligonucleotides of (10).

ACTIVITY - Neuroprotective; antiinflammatory; anti-HIV (human immunodeficiency virus); antidiabetic; anorectic;  
antibacterial; antiarteriosclerotic; cytostatic. No biological data is  
given.

MECHANISM OF ACTION - ATP-mediated 1-phosphorylation of ceramides.

USE - For treatment and prevention of disorders including  
neurological disease, inflammation, human immunodeficiency  
virus (HIV) infection, diabetes (type 2), obesity, sepsis,  
arteriosclerosis and cancer.

EXAMPLE - The NCBI expressed sequence tag (EST) database  
is searched using the tblastn algorithm for homologies to mouse  
sphingosine kinase 1. A tag (GenBank AA355581) is identified and primes  
synthesized enclosing it. These are used for polymerase chain reaction  
amplification of a cDNA library from human leukemia cells (Clontech). The amplification fragment is used to screen a colonic  
cDNA library in lambda ZAP-II phage. Clones of 1.5 kbase, 3.5  
kbase and 4.4 kbase are isolated and sequenced. Together these encode the  
sequence of a new ceramide kinase (CERK1). This is  
inserted into pCR3.1 vector (Invitrogen) and used to transform HEK293  
cells (ATCC CRL-1573). The phosphorylation activity of a culture of these  
cells using N-hexanoyl-D-erythro-sphingosine as substrate is 1280  
pmol/minute/mg compared to 7 pmol/minute/mg for cells transformed by  
pCR3.1 vector only. (61 pages)

L5 ANSWER 21 OF 22 MEDLINE on STN

ACCESSION NUMBER: 1999233575 MEDLINE

DOCUMENT NUMBER: PubMed ID: 10216276

TITLE: Signalling sphingomyelinases: which, where, how and why?.

AUTHOR: Levade T; Jaffrezou J P

CORPORATE SOURCE: INSERM Unit 466, Laboratoire de Biochimie, Maladies

Metaboliques, Institut Louis Bugnard, Bat. L3, C.H.U.  
 Rangueil, 1 Avenue Jean Poulhes, E 9910, Toulouse Cedex 4,  
 France.. levade@rangueil.inserm.fr  
 SOURCE: Biochimica et biophysica acta, (1999 Apr 19) 1438 (1) 1-17.  
 Ref: 186  
 Journal code: 0217513. ISSN: 0006-3002.  
 PUB. COUNTRY: Netherlands  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 General Review; (REVIEW)  
 (REVIEW, TUTORIAL)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199906  
 ENTRY DATE: Entered STN: 19990614  
 Last Updated on STN: 19990614  
 Entered Medline: 19990602

AB A major lipid signalling pathway in mammalian cells implicates the activation of sphingomyelinase (SMase), which upon cell stimulation hydrolyses the ubiquitous sphingophospholipid sphingomyelin to ceramide. This review summarizes our current knowledge on the nature and regulation of signalling SMase(s). Because of the controversy on the identity of this(these) phospholipase(s), the roles of various SMases in cell signalling are discussed. Special attention is also given to the subcellular site of action of signalling SMases and to the cellular factors that positively or negatively control their activity. These regulating agents include lipids (arachidonic acid, diacylglycerol and ceramide), kinases, proteases, glutathione and other proteins.

L5 ANSWER 22 OF 22 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1994:187156 SCISEARCH  
 THE GENUINE ARTICLE: NC811  
 TITLE: CERAMIDE DOES NOT MEDIATE THE EFFECT OF  
 TUMOR-NECROSIS-FACTOR-ALPHA ON SUPEROXIDE GENERATION IN  
 HUMAN NEUTROPHILS  
 AUTHOR: YANAGA F (Reprint); WATSON S P  
 CORPORATE SOURCE: UNIV OXFORD, DEPT PHARMACOL, MANSFIELD RD, OXFORD OX1 3QT,  
 ENGLAND (Reprint)  
 COUNTRY OF AUTHOR: ENGLAND  
 SOURCE: BIOCHEMICAL JOURNAL, (15 MAR 1994) Vol. 298, Part 3, pp.  
 733-738.  
 ISSN: 0264-6021.  
 PUBLISHER: PORTLAND PRESS, 59 PORTLAND PLACE, LONDON, ENGLAND W1N 3AJ

DOCUMENT TYPE: Article; Journal  
 FILE SEGMENT: LIFE  
 LANGUAGE: English  
 REFERENCE COUNT: 30  
 ENTRY DATE: Entered STN: 1994  
 Last Updated on STN: 1994

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The effect of tumour necrosis factor alpha (TNFalpha) on superoxide generation in human neutrophils was investigated using the Nitro Blue Tetrazolium reduction assay. TNFalpha stimulated superoxide generation in a time- and concentration-dependent fashion. The maximally effective concentration of TNFalpha for superoxide generation was 10 nM and maximal response was obtained after 15-20 min. The monoclonal antibody (mAb), utr-1, which was raised against the 75 kDa receptor and behaves as an antagonist, had no effect on superoxide generation, but partially inhibited the response to TNFalpha. mAb htr-9, which was raised against the 55 kDa receptor and behaves as an agonist, mimicked the effect of TNFalpha, but with a lower maximal response. As it has been reported that ceramide might act as a second messenger to mediate many of the effects of TNFalpha, the effects of exous C2-sphingomyelinase and the cell-permeable ceramide analogue, C2-ceramide, on production of superoxide anions, induction of priming in response to formylmethionyl-leucyl-phenylalanine, and cell-shape change were examined. Neither sphingomyelinase nor C2-ceramide mimicked the effect of TNFalpha.

Ceramide is converted into ceramide 1-phosphate by ceramide kinase and we have measured levels of this metabolite to clarify the effect of TNFalpha on sphingomyelinase activity in neutrophils. Although exogenous sphingomyelinase increased the amount of ceramide 1-phosphate in a time-dependent manner, and C2-ceramide was rapidly converted into C2-ceramide phosphate, TNFalpha had no effect on the level of ceramide 1-phosphate. These results suggest that TNFalpha stimulates superoxide generation through both the 55 kDa and 75 kDa receptors, but that ceramide does not act as an intracellular mediator for TNFalpha in human neutrophils.

=> d his

(FILE 'HOME' ENTERED AT 12:22:25 ON 13 SEP 2005)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 12:22:51 ON 13 SEP 2005

L1 181 S CERAMIDE (W) KINASE?  
L2 99 S HUMAN AND L1  
L3 7258991 S CLON? OR EXPRESS? OR RECOMBINANT  
L4 49 S L2 AND L3  
L5 22 DUP REM L4 (27 DUPLICATES REMOVED)

=> s shingosine (w)kinase?

L6 2 SHINGOSINE (W) KINASE?

=> s sphingosine (w)kinase?

L7 2026 SPHINGOSINE (W) KINASE?

=> s homolog? or identit?

L8 1467318 HOMOLOG? OR IDENTIT?

=> s human nd l8

MISSING OPERATOR ND L8

The search profile that was entered contains terms or nested terms that are not separated by a logical operator.

=> s human and l8

L9 526525 HUMAN AND L8

=> l and l9

L IS NOT A RECOGNIZED COMMAND

The previous command name entered was not recognized by the system. For a list of commands available to you in the current file, enter "HELP COMMANDS" at an arrow prompt (=>).

=> s l7 and l9

L10 99 L7 AND L9

=> dup rem l10

PROCESSING COMPLETED FOR L10

L11 47 DUP REM L10 (52 DUPLICATES REMOVED)

=> d 1-47 ibib ab

L11 ANSWER 1 OF 47 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2005:394682 HCAPLUS

DOCUMENT NUMBER: 142:445550

TITLE: Gene expression profiles for the diagnosis and prognosis of breast cancer

INVENTOR(S): Erlander, Mark; Ma, Xiao-Jun; Wang, Wei; Wittliff, James L.

PATENT ASSIGNEE(S): Arcturus Bioscience, Inc. University of Louisville, USA

SOURCE: U.S. Pat. Appl. Publ., 40 pp.

CODEN: USXXCO

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2005095607	A1	20050505	US 2004-795092	20040305
PRIORITY APPLN. INFO.:			US 2003-453006P	P 20030307

AB The invention relates to the identification and use of gene expression profiles, or patterns, suitable for identification of breast cancer patient populations with different survival outcomes. The gene expression profiles may be embodied in nucleic acid expression, protein expression, or other expression formats, and may be used in the study and/or determination of the prognosis of a patient, including breast cancer survival.

L11 ANSWER 2 OF 47 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2005:671727 HCAPLUS

DOCUMENT NUMBER: 143:166667

TITLE: The curcuminoids- and anthocyanins-responsive genes in human adipocytes and their use in screenings of anti-obesity and anti-diabetes drugs

INVENTOR(S): Ueno, Yuki; Tsuda, Takanori; Takanori, Hitoshi; Yoshikawa, Toshikazu; Osawa, Toshihiko

PATENT ASSIGNEE(S): Biomarker Science Co., Ltd., Japan

SOURCE: Jpn. Kokai Tokkyo Koho, 85 pp.

CODEN: JKXXAF

DOCUMENT TYPE: Patent

LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2005198640	A2	20050728	JP 2004-53258	20040227
PRIORITY APPLN. INFO.:			JP 2003-394758	A 20031125

AB The curcuminoids- and anthocyanins-responsive gene expression profiles in adipocytes have been revealed. The curcuminoids- and anthocyanins-responsive genes are designed to be used as the index markers in the screenings of the substances that can affect the gene expression patterns in obesity and diabetes. These substances can be the candidates of anti-obesity and anti-diabetes drugs. Therefore, the groups of curcuminoids- and anthocyanins-responsive genes are intended to be used as markers in a form of kit such as DNA chip for the screening of anti-obesity and anti-diabetes drugs.

L11 ANSWER 3 OF 47 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2005:549535 HCAPLUS

TITLE: Building a human kinase gene repository: Bioinformatics, molecular cloning, and functional validation

AUTHOR(S): Park, Jaehong; Hu, Yanhui; Murthy, T. V. S.; Vannberg, Fredrik; Shen, Binghua; Rolfs, Andreas; Hutti, Jessica E.; Cantley, Lewis C.; LaBaer, Joshua; Harlow, Ed; Brizuela, Leonardo

CORPORATE SOURCE: Harvard Institute of Proteomics, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Cambridge, MA, 02141, USA

SOURCE: Proceedings of the National Academy of Sciences of the United States of America (2005), 102(23), 8114-8119  
CODEN: PNASA6; ISSN: 0027-8424

PUBLISHER: National Academy of Sciences

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Kinases catalyze the phosphorylation of proteins, lipids, sugars, nucleosides, and other important cellular metabolites and play key regulatory roles in all aspects of eukaryotic cell physiolo. Here, we describe the mining of public databases to collect the sequence information of all identified human kinase genes and the cloning of the corresponding ORFs. We identified 663 genes, 511 encoding protein kinases, and 152 encoding nonprotein kinases. We describe the successful

cloning and sequence verification of 270 of these genes. Subcloning of this gene set in mammalian expression vectors and their use in high-throughput cell-based screens allowed the validation of the clones at the level of expression and the identification of previously uncharacterized modulators of the survivin promoter. Moreover, expressions of the kinase genes in bacteria, followed by autophosphorylation assays, identified 21 protein kinases that showed autocatalytic activity. The work described here will facilitate the functional assaying of this important gene family in phenotypic screens and their use in biochem. and structural studies.

REFERENCE COUNT: 45 THERE ARE 45 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 4 OF 47 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.  
on STN

ACCESSION NUMBER: 2005261082 EMBASE  
TITLE: The ins and outs of sphingolipid synthesis.  
AUTHOR: Futerman A.H.; Riezman H.  
CORPORATE SOURCE: A.H. Futerman, Department of Biological Chemistry, Weizmann  
Institute of Science, Rehovot 76100, Israel.  
tony.futerman@weizmann.ac.il  
SOURCE: Trends in Cell Biology, (2005) Vol. 15, No. 6, pp. 312-318.  
Refs: 59  
ISSN: 0962-8924 CODEN: TCBIK  
PUBLISHER IDENT.: S 0962-8924(05)00107-8  
COUNTRY: United Kingdom  
DOCUMENT TYPE: Journal; General Review  
FILE SEGMENT: 029 Clinical Biochemistry  
LANGUAGE: English  
SUMMARY LANGUAGE: English  
ENTRY DATE: Entered STN: 20050714  
Last Updated on STN: 20050714

AB Sphingolipids are ubiquitous components of eukaryotic cell membranes, where they play important roles in intracellular signaling and in membrane structure. Even though the biochemical pathway of sphingolipid synthesis and its compartmentalization between the endoplasmic reticulum and Golgi apparatus have been known for many years, the molecular identity of the enzymes in this pathway has only recently been elucidated. Here, we summarize progress in the identification and characterization of the enzymes, the transport of ceramide from the endoplasmic reticulum to the Golgi apparatus, and discuss how regulating the synthesis of sphingolipids might impact upon their functions. .COPYRG. 2005 Elsevier Ltd. All rights reserved.

L11 ANSWER 5 OF 47 MEDLINE on STN DUPLICATE 1

ACCESSION NUMBER: 2005016608 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 15643073  
TITLE: Sphingosine kinase regulates the  
sensitivity of Dictyostelium discoideum cells to the  
anticancer drug cisplatin.  
AUTHOR: Min Junxia; Traynor David; Stegner Andrew L; Zhang Lei;  
Hanigan Marie H; Alexander Hannah; Alexander Stephen  
CORPORATE SOURCE: Division of Biological Sciences, University of Missouri,  
Columbia, MO 65211-7400, USA.  
CONTRACT NUMBER: CA57530 (NCI)  
CA95872 (NCI)  
GM53929 (NIGMS)  
SOURCE: Eukaryotic cell, (2005 Jan) 4 (1) 178-89.  
Journal code: 101130731. ISSN: 1535-9778.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200505  
ENTRY DATE: Entered STN: 20050112  
Last Updated on STN: 20050525  
Entered Medline: 20050524

AB The drug cisplatin is widely used to treat a number of tumor types. However, resistance to the drug, which remains poorly understood, limits

its usefulness. Previous work using Dictyostelium discoideum as a model for studying drug resistance showed that mutants lacking sphingosine-1-phosphate (S-1-P) lyase, the enzyme that degrades S-1-P, had increased resistance to cisplatin, whereas mutants overexpressing the enzyme were more sensitive to the drug. S-1-P is synthesized from sphingosine and ATP by the enzyme sphingosine kinase. We have identified two sphingosine kinase genes in D. discoideum--sgkA and sgkB--that are homologous to those of other species. The biochemical properties of the SgkA and SgkB enzymes suggest that they are the equivalent of the human Sphk1 and Sphk2 enzymes, respectively. Disruption of the kinases by homologous recombination (both single and double mutants) or overexpression of the sgkA gene resulted in altered growth rates and altered response to cisplatin. The null mutants showed increased sensitivity to cisplatin, whereas mutants overexpressing the sphingosine kinase resulted in increased resistance compared to the parental cells. The results indicate that both the SgkA and the SgkB enzymes function in regulating cisplatin sensitivity. The increase in sensitivity of the sphingosine kinase-null mutants was reversed by the addition of S-1-P, and the increased resistance of the sphingosine kinase overexpressor mutant was reversed by the inhibitor N,N-dimethylsphingosine. Parallel changes in sensitivity of the null mutants are seen with the platinum-based drug carboplatin but not with doxorubicin, 5-fluorouracil, and etoposide. This pattern of specificity is similar to that observed with the S-1-P lyase mutants and should be useful in designing therapeutic schemes involving more than one drug. This study identifies the sphingosine kinases as new drug targets for modulating the sensitivity to platinum-based drugs.

L11 ANSWER 6 OF 47 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2005:724003 SCISEARCH

THE GENUINE ARTICLE: 942FZ

TITLE: Loss of heterozygosity and transcriptome analyses of a 1.2 Mb candidate ovarian cancer tumor suppressor locus region at 17q25.1-q25.2

AUTHOR: Presneau N; Dewar K; Forgetta V; Provencher D; Mes-Masson A M; Tonin P N (Reprint)

CORPORATE SOURCE: Montreal Gen Hosp, Room L10-120, 1650 Cedar Ave, Montreal, PQ H3G 1A4, Canada (Reprint); McGill Univ, Ctr Hlth, Res Inst, Montreal, PQ, Canada; McGill Univ, Dept Human Genet, Montreal, PQ, Canada; McGill Univ, Genome Quebec Innovat Ctr, Montreal, PQ, Canada; Univ Montreal, Hop Notre Dame, Ctr Hosp, Ctr Rech, Montreal, PQ, Canada; Univ Montreal, Inst Canc Montreal, Montreal, PQ H2L 4M1, Canada; Univ Montreal, Dept Obstet Gynecol, Montreal, PQ, Canada; Univ Montreal, Dept Med, Montreal, PQ H3C 3J7, Canada; McGill Univ, Dept Med, Montreal, PQ, Canada

COUNTRY OF AUTHOR: Canada

SOURCE: MOLECULAR CARCINOGENESIS, (JUL 2005) Vol. 43, No. 3, pp. 141-154.

ISSN: 0899-1987.

PUBLISHER: WILEY-LISS, DIV JOHN WILEY & SONS INC, 111 RIVER ST, HOBOKEN, NJ 07030 USA.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 60

ENTRY DATE: Entered STN: 22 Jul 2005

Last Updated on STN: 22 Jul 2005

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Loss of heterozygosity (LOH) analysis was performed in epithelial ovarian cancers (EOC) to further characterize a previously identified candidate tumor suppressor gene (TSG) region encompassing D17S801 at chromosomal region 17q25.1. LOH of at least one informative marker was observed for 100 (71%) of 140 malignant EOC samples in an analysis of 6 polymorphic markers (cen-D17S1839-D17S785-D17S1817-D17S801-D17S751-D17S722-tel). The combined LOH analysis revealed a 453 kilobase (Kb) minimal region of deletion (MRD) bounded by D17S1817 and D17S751. Human and mouse genome assemblies were used to resolve marker inconsistencies in



the D17S1839-D17S722 interval and identify candidates. The region contains 32 known and strongly predicted genes, 9 of which overlap the MRD. The reference genomic sequences share nearly identical gene structures and the organization of the region is highly collinear. Although, the region does not show any large internal duplications, a 1.5 Kb inverted duplicated sequence of 87% nucleotide identity was observed in a 13 Kb region surrounding D17S801. Transcriptome analysis by Affymetrix GeneChip((R)) and reverse transcription (RT)-polymerase chain reaction (PCR) methods of 3 well characterized EOC cell lines and primary cultures of normal ovarian surface epithelial (NOSE) cells was performed with 32 candidates spanning D17S1839-D17S722 interval. RT-PCR analysis of 8 known or strongly predicted genes residing in the MRD in 10 EOC samples, that exhibited LOH of the MRD, identified FLJ22347 as a strong candidate TSG. The proximal repeat sequence of D17S801 occurs 8 Kb upstream of the putative promoter region of FLJ22341. RT-PCR analysis of the EOC samples and cell lines identified DKFZP434P0316 that maps proximal to the MRD, as a candidate. While Affymetrix technology was useful for initially eliminating less promising candidates, subsequent RT-PCR analysis of well-characterized EOC samples was essential to prioritize TSG candidates for further study. (c) 2005 Wiley-Liss, Inc.

L11 ANSWER 7 OF 47 MEDLINE on STN  
 ACCESSION NUMBER: 2005119749 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 15750338  
 TITLE: RPK118, a PX domain-containing protein, interacts with peroxiredoxin-3 through pseudo-kinase domains.  
 AUTHOR: Liu Lingling; Yang Chenyi; Yuan Jian; Chen Xiujuan; Xu Jianing; Wei Youheng; Yang Jingchun; Lin Gang; Yu Long  
 CORPORATE SOURCE: State Key Laboratory of Genetic Engineering, Institute of Genetics, School of Life Science, Fudan University, Shanghai 200433, China.  
 SOURCE: Molecules and cells, (2005 Feb 28) 19 (1) 39-45.  
 Journal code: 9610936. ISSN: 1016-8478.  
 PUB. COUNTRY: Korea (South)  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200507  
 ENTRY DATE: Entered STN: 20050308  
 Last Updated on STN: 20050729  
 Entered Medline: 20050728

AB RPK118 is a sphingosine kinase-1-binding protein that has been implicated in sphingosine 1 phosphate-mediated signaling. It contains a PX (phox homology) domain and two pseudo-kinase domains, and co-localizes with sphingosine kinase-1 on early endosomes. In this study we identified a novel RPK118-binding protein, PRDX3 (peroxiredoxin-3), by yeast two-hybrid screening. The interaction between these proteins was confirmed by pull-down assays and co-immunoprecipitation experiments. Deletion studies showed that RPK118 interacted with PRDX3 through its pseudokinase domains, and with early endosomes through its PX domain. Double immunofluorescence experiments demonstrated that PRDX3 co-localized with RPK118 on early endosomes in COS7 cells. PRDX3 is a member of the antioxidant family of proteins synthesized in the cytoplasm and functioning in mitochondria. Our findings indicate that RPK118 is a PRDX3-binding protein that may be involved in transporting PRDX3 from the cytoplasm to its mitochondrial site of function or to other membrane structures via endosome trafficking.

L11 ANSWER 8 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN  
 ACCESSION NUMBER: 2004-14417 BIOTECHDS  
 TITLE: Modulating mammalian endothelial cell functional characteristics such as viability, proliferation and differentiation, useful for treating tumor, rheumatoid arthritis, involves modulating functional level of sphingosine kinase;  
 useful for preparation of a medicament for gene therapy  
 AUTHOR: GAMBLE J R; VADAS M; PITSON S; XIA P; LIMAYE V  
 PATENT ASSIGNEE: MEDVET SCI PTY LTD  
 PATENT INFO: WO 2004035786 29 Apr 2004

APPLICATION INFO: WO 2003-AU1356 14 Oct 2003  
PRIORITY INFO: AU 2003-902047 30 Apr 2003; AU 2002-952032 14 Oct 2002  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
OTHER SOURCE: WPI: 2004-365161 [34]

AB DERWENT ABSTRACT:

NOVELTY - Modulating (M1) one or more mammalian endothelial cell functional characteristics, involves modulating the functional level of **sphingosine kinase**, where inducing over-expression of the **sphingosine kinase** level modulates one or more of the functional characteristics of the endothelial cell.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) use of an agent capable of modulating the functional level of **sphingosine kinase** in the manufacture of a medicament for (M1); (2) use of **sphingosine kinase** or a nucleic acid encoding **sphingosine kinase** in the manufacture of a medicament for (M1); and (3) a pharmaceutical composition comprising modulatory agent and one or more carriers and/or diluents when used in (M1).

WIDER DISCLOSURE - The following are disclosed: (1) generating endothelial cells by (M1); and (2) endothelial cells generated by (M1).

BIOTECHNOLOGY - Preferred Method: In (M1), the endothelial cell is a vascular endothelial cell. The endothelial cell functional characteristic is up-regulatable by **sphingosine kinase** over-expression and the characteristic is one or more of viability, proliferation, differentiation, cell surface molecule expression, cytokine responsiveness or enhanced proliferation or viability. The cell surface molecule is an adhesion molecule. The functional characteristic is up-regulated. The endothelial cell functional characteristic is up-regulatable by **sphingosine kinase** over-expression and the characteristic is the induction of a pro-inflammatory phenotype or angiogenic phenotype or maintenance of the CD34+ endothelial cell progenitor phenotype. The pro-inflammatory phenotype is down-regulated. The angiogenic phenotype is up-regulated or down-regulated. The CD34+ progenitor phenotype is maintained. The modulation is up-regulation of **sphingosine kinase** levels and the up-regulation is achieved by introducing into the endothelial cell a nucleic acid molecule encoding **sphingosine kinase** or its functional equivalent, derivative or homologue or the **sphingosine kinase** expression product or its functional derivative, homologue, analogue, equivalent or mimetic. The modulation is achieved by contacting the endothelial cell with a proteinaceous or non-proteinaceous molecule which modulates transcriptional and/or translational regulation of the **sphingosine kinase** gene. Modulation is up-regulation of **sphingosine kinase** levels and the up-regulation is achieved by contacting the endothelial cell with a proteinaceous or non-proteinaceous molecule, which functions as an agonist of the **sphingosine kinase** expression product. The modulation is down-regulation of **sphingosine kinase** levels and the down-regulation is achieved by contacting the endothelial cell with a proteinaceous or non-proteinaceous molecule which functions as an antagonist to the **sphingosine kinase** expression product. The molecule is a mutant **sphingosine kinase** which mutant is characterized by substitution of the glycine residue at position 82 to aspartate. The endothelial cell activity is modulated in vivo or in vitro. In the method of using an agent capable of modulating **sphingosine kinase** in the manufacture of medicament, the agent is a proteinaceous or non-proteinaceous molecule, which modulates transcriptional and/or translational regulation of the **sphingosine kinase** gene, functions as an agonist of **sphingosine kinase** activity or functions as an antagonist of **sphingosine kinase** activity.

ACTIVITY - Vulnerary; Antiarthritic; Antirheumatic; Cytostatic; Antiangiogenic. No biological data given.

MECHANISM OF ACTION - Protein Kinase Modulator; **Sphingosine Kinases** Modulator; Gene Therapy. Adenovirus carrying the **sphingosine kinase** (SK) gene were used to transfect vascular endothelial cells. Overexpression of SK was measured and found

to be increased by 5.17-fold. Use of DAPI stain under basal conditions and under serum deprivation conditions showed that cells overexpressing SK were less likely to undergo apoptosis. Caspase-3 activity was also measured and found to be suppressed under higher SK levels.

**USE** - For modulating mammalian endothelial cell functions such as viability, proliferation, differentiation, cell surface molecule expression, cytokine responsiveness or enhanced proliferation or viability. (M1) is also useful for prophylaxis and/or treatment of a condition characterized by aberrant or otherwise unwanted endothelial cell functioning in a mammal. The medicament manufactured using agent capable of modulating the functional level of **sphingosine kinase** or a nucleic acid encoding **sphingosine kinase**, is useful for treating a condition characterized by aberrant or otherwise unwanted endothelial cell functioning in a mammal. The condition is vascular engraftment, wound repair, tissue or organ transplantation or the repair of devascularised tissue and the modulated endothelial cell functional characteristic is one or more of enhanced endothelial cell proliferation, enhanced endothelial cell viability or maintenance of the CD34+ progenitor phenotype. The condition is an inflammatory condition and the modulated endothelial cell functional characteristic is down-regulation of one or more of an endothelial cell inflammatory or angiogenic phenotype. The condition is rheumatoid arthritis. The condition is characterized by unwanted angiogenesis and the modulated endothelial cell functional characteristic is down-regulation of an endothelial cell angiogenic phenotype. The condition is a tumor (all claimed).

**ADMINISTRATION** - Administration of the modulatory agent is by oral, intravenous, intramuscular, intraperitoneal, subcutaneous, intradermal, suppository routes or implanting (e.g., using slow release molecules) at 0.1-1 mg/kg body weight/day.

**EXAMPLE** - To determine the effect on endothelial cell function of over-expression of **sphingosine kinase** (SK), HUVEC (human vascular endothelial cells) were infected with either retrovirus containing SK or adenovirus containing SK, at 1 plate forming units (pfu)/cell. This level of adenovirus infection was chosen since it resulted in similar levels of SK activity as tumor necrosis factor (TNF) alpha-stimulation of endogenous SK in endothelial cells, and similar levels of SK activity as was achieved with retrovirus-mediated gene delivery. To determine whether over-expression of SK results in changes to the endogenous phenotype of endothelial cells, the adhesion molecule expression was investigated on these infected cells. Retrovirus-mediated over-expression of SK up-regulated basal VCAM-1 expression. Adenoviral-mediated over-expression of SK resulted in a similar increase in VCAM-1 expression. In contrast to VCAM-1, basal E selectin expression was not altered in cells over-expressing SK generated by retroviral or adenoviral-mediated transfection. As over-expression of SK induced basal levels of VCAM-1. To determine whether these cells exhibited an altered response to stimulation with TNFalpha-induced up-regulation of VCAM-1 expression. Interestingly, cells over-expressing SK also showed an enhanced E Selectin response following stimulation with TNFalpha even though basal E Selectin expression was not altered. Over-expression of dominant-negative SK (G82D) significantly inhibited the induction of VCAM-1 and E Selectin in response to TNFalpha compared with empty vector (EV). Significant levels of both adhesion molecules were induced in cells over-expressing SK. Retroviral and adenoviral delivery of SK generated similar phenotypes in endothelial cells, that of enhanced expression of adhesion molecules and altered response to TNFalpha. However the adenoviral system enabled large number of cells to be rapidly generated. To determine whether the alteration in adhesion molecule expression resulting from intracellular over-expression of SK had functional consequences, neutrophil adhesion to endothelial cells was measured. In the basal state, cells over-expressing SK showed significant neutrophil adhesion, in contrast to control cells which did not bind neutrophils. Stimulation of endothelial cells with a low dose of TNFalpha (0.04 ng/ml) resulted in minimal neutrophil adhesion in control cells, but significantly greater adhesion to cells over-expressing SK. Consistent with a role for SK in mediating PMN adhesion, endothelial cells over-expressing the dominant-negative SK, G82D, inhibited PMN adhesion in response to stimulation with TNF alpha. To determine whether SK

over-expression also enhances the ability of endothelial cells to form tubes. Endothelial cells were plated onto the complex basement membrane matrix, Matrigel, Equivalent numbers of cells over-expressing SK and EV were seeded, cells over-expressing SK had already commenced realignment whereas the EV cells remained disorganized. By 30 minutes cells over-expressing SK showed greater evidence of tube alignment compared with EV cells. By one hour tube formation by cells over-expressing SK was highly developed compared with EV cells. By 18 hours, a time where tube formation was complete, both cells over-expressing SK and EV cells showed a similar pattern of tube formation. These results suggest that over-expression of SK stimulates the rate of tube formation. (91 pages)

L11 ANSWER 9 OF 47 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2004:565044 HCAPLUS

DOCUMENT NUMBER: 141:104809

TITLE: Differentially expressed human nucleic acid and proteins for diagnosis and treatment of cancer  
INVENTOR(S): Lightcap, Eric S.; Ecsedy, Jeffrey A.; Hunter, John J.; Macbeth, Kyle J.; Nestor, Michelle Tighe

PATENT ASSIGNEE(S): Millennium Pharmaceuticals, Inc., USA

SOURCE: PCT Int. Appl., 576 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004058153	A2	20040715	WO 2003-US40226	20031216
W:			AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW	
RW:			BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG	
US 2004235071	A1	20041125	US 2003-737450	20031216
PRIORITY APPLN. INFO.:			US 2002-435108P	P 20021220
			US 2002-436443P	P 20021223
			US 2003-438498P	P 20030107
			US 2003-444370P	P 20030131
			US 2003-446031P	P 20030206
			US 2003-453635P	P 20030311
			US 2003-457199P	P 20030325
			US 2003-462458P	P 20030410
			US 2003-466732P	P 20030430
			US 2003-469184P	P 20030508
			US 2003-471663P	P 20030519
			US 2003-475472P	P 20030603
			US 2003-478150P	P 20030612
			US 2003-480631P	P 20030623
			US 2003-487369P	P 20030715
			US 2003-490866P	P 20030729
			US 2003-499614P	P 20030902
			US 2003-510081P	P 20031009
			US 2003-517742P	P 20031106

AB The present invention relates to methods for the diagnosis and treatment of a cancer or cancer. Specifically, the present invention identifies the differential expression of 72 genes in tissues relating to cancer, relative to their expression in normal, or non-cancer disease states, and/or in response to manipulations relevant to a cancer, as determined by Taqman anal. and in situ hybridization. The present invention describes methods for the diagnostic evaluation and prognosis of various cancers, and for the identification of subjects exhibiting a predisposition to such conditions. The invention also provides methods for identifying a compound

capable of modulating a cancer or cancer. The present invention also provides methods for the identification and therapeutic use of compds. as treatments of cancer.

L11 ANSWER 10 OF 47 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2005:60760 HCAPLUS  
Correction of: 2004:1036573

DOCUMENT NUMBER: 142:153477  
Correction of: 142:16776

TITLE: Gene expression profiles and biomarkers for the detection of Chagas disease and other disease-related gene transcripts in blood

INVENTOR(S): Liew, Choong-Chin

PATENT ASSIGNEE(S): Chondrogene Limited, Can.

SOURCE: U.S. Pat. Appl. Publ., 154 pp., Cont.-in-part of U.S. Ser. No. 802,875.  
CODEN: USXXCO

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 46

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2004241729	A1	20041202	US 2004-813097	20040330
US 2004014059	A1	20040122	US 2002-268730	20021009
US 2005191637	A1	20050901	US 2004-803737	20040318
US 2005196762	A1	20050908	US 2004-803759	20040318
US 2005196763	A1	20050908	US 2004-803857	20040318
US 2005196764	A1	20050908	US 2004-803858	20040318
US 2004241729	A1	20041202	US 2004-813097	20040330
US 2004241729	A1	20041202	US 2004-813097	20040330
US 2004265869	A1	20041230	US 2004-812716	20040330
PRIORITY APPLN. INFO.:			US 1999-115125P	P 19990106
			US 2000-477148	B1 20000104
			US 2002-268730	A2 20021009
			US 2003-601518	A2 20030620
			US 2004-802875	A2 20040312
			US 2004-813097	A 20040330

AB The present invention is directed to detection and measurement of gene transcripts and their equivalent nucleic acid products in blood. Specifically provided is anal. performed on a drop of blood for detecting, diagnosing, and monitoring diseases, and in particular Chagas disease, using gene-specific and/or tissue-specific primers. Affymetrix Human Genome U133 and ChondroChip microarrays were used to detect differentially expressed gene transcripts in hypertension, obesity, allergy, systemic steroids, coronary artery disease, diabetes type 2, hyperlipidemia, lung disease, bladder cancer, rheumatoid arthritis, osteoarthritis, liver cancer, schizophrenia, Chagas disease, asthma, and manic depression syndrome. The present invention describes methods by which delineation of the sequence and/or quantitation of the expression levels of disease-specific genes allows for an immediate and accurate diagnostic/prognostic test for disease or to assess the effect of a particular treatment regimen. [This abstract record is one of 3 records for this document necessitated by the large number of index entries required to fully index the document and publication system constraints.].

L11 ANSWER 11 OF 47 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2004:1019619 HCAPLUS

DOCUMENT NUMBER: 142:1802

TITLE: Japanese macaque herpesvirus nucleic acid and polypeptide sequences and their use in disease model for multiple sclerosis and for screening therapeutic agents

INVENTOR(S): Wong, Scott W.; Axthelm, Michael K.; Hansen, Scott G.

PATENT ASSIGNEE(S): Oregon Health & Science University, USA

SOURCE: U.S. Pat. Appl. Publ., 250 pp., Cont.-in-part of U.S. Ser. No. 276,524.  
CODEN: USXXCO

DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 2  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2004234953	A1	20041125	US 2004-779597	20040212
WO 2001088203	A1	20011122	WO 2001-US16274	20010517

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.:  
US 2000-205652P P 20000518  
WO 2001-US16274 W 20010517  
US 2002-276524 A2 20021113

AB Japanese macaques can harbor a virus related to Rhesus macaque rhadinovirus, called Japanese macaque herpesvirus (JMHV), is harbored in inflamed spinal cord lesions obtained from a Japanese macaque monkey with spontaneous multiple sclerosis-like disease. An isolated virus is disclosed herein (Japanese macaque herpesvirus, JMHV), as are viral particles including this virus and host cells infected with this virus. The entire nucleic acids sequence of this virus is provided herein. Also disclosed are the nucleic acid sequences of unique open reading frames, and the 171 polypeptide sequences encoded by these open reading frames. Pharmaceutical compns. are also disclosed that include the viral nucleic acid, a polypeptide encoded by the viral nucleic acid, an antibody that binds the JMHV polypeptide, or a polynucleotide encoding at least one JMHV polypeptide. Model systems for screening for agents of use in the treatment of multiple sclerosis are also disclosed.

L11 ANSWER 12 OF 47 MEDLINE on STN  
ACCESSION NUMBER: 2004612131 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 15585953  
TITLE: Identification of genetic and epigenetic similarities of SPHK1/Sphk1 in mammals.  
AUTHOR: Imamura Takuya; Miyauchi-Senda Nanami; Tanaka Satoshi; Shiota Kunio  
CORPORATE SOURCE: Laboratory of Cellular Biochemistry, Animal Resource Sciences, Veterinary Medical Sciences, The University of Tokyo, Japan.  
SOURCE: Journal of veterinary medical science / the Japanese Society of Veterinary Science, (2004 Nov) 66 (11) 1387-93. Journal code: 9105360. ISSN: 0916-7250.  
PUB. COUNTRY: Japan  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200501  
ENTRY DATE: Entered STN: 20041209  
Last Updated on STN: 20050126  
Entered Medline: 20050125

AB In normal tissues, methylation of CpG islands is generally accepted to be limited to the inactive X-chromosome and imprinting clusters. Gene Sphk1 has shown complex organization, indicated by multiple alternative splicing and tissue-dependent DNA methylation within the limited area (T-DMR) of the CpG island in the rat. Comparisons among human, mouse and rat SPHK1/Sphk1 genomic DNA revealed five coding exons and association of a CpG island at the 5' end in common. We also found two novel subtypes, for a total of eight mRNA subtypes generated through selective usage of untranslated first exons. A 38-bp region at the 5'-end of T-DMR is highly conserved. This restricted area is specifically hypomethylated in the brain. Here, we examine the complex genetic/epigenetic features of the SPHK1/Sphk1 CpG island, and suggest that the T-DMR is the core target for

tissue-dependent CpG island methylation.

L11 ANSWER 13 OF 47 MEDLINE on STN DUPLICATE 2  
ACCESSION NUMBER: 2004165949 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 15059942  
TITLE: Point-counterpoint of sphingosine 1-phosphate metabolism.  
AUTHOR: Saba Julie D; Hla Timothy  
CORPORATE SOURCE: Children's Hospital of Oakland Research Institute, Oakland, Calif, USA.  
CONTRACT NUMBER: CA77528 (NCI)  
GM66954 (NIGMS)  
HL67330 (NHLBI)  
HL70694 (NHLBI)  
SOURCE: Circulation research, (2004 Apr 2) 94 (6) 724-34. Ref: 113  
Journal code: 0047103. ISSN: 1524-4571.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
General Review; (REVIEW)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200407  
ENTRY DATE: Entered STN: 20040403  
Last Updated on STN: 20040717  
Entered Medline: 20040716

AB Sphingosine 1-phosphate (S1P), an evolutionarily conserved bioactive lipid mediator, is now recognized as a potent modulator of cell regulation. In vertebrates, S1P interacts with cell surface G protein-coupled receptors of the EDG family and induces profound effects in a variety of organ systems. Indeed, an S1P receptor agonist is undergoing clinical trials to combat immune-mediated transplant rejection. Recent information on S1P receptor biology suggests potential utility in the control of cardiovascular processes, including angiogenesis, vascular permeability, arteriogenesis, and vasospasm. However, studies from diverse invertebrates, such as yeast, Dictyostelium, Drosophila, and Caenorhabditis elegans have shown that S1P is involved in important regulatory functions in the apparent absence of EDG S1P receptor homologues. Metabolic pathways of S1P synthesis, degradation, and release have recently been described at the molecular level. Genetic and biochemical studies of these enzymes have illuminated the importance of S1P signaling systems both inside and outside of cells. The revelation of receptor-dependent pathways, as well as novel metabolic/intracellular pathways has provided new biological insights and may ultimately pave the way for the development of novel therapeutic approaches for cardiovascular diseases.

L11 ANSWER 14 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN  
ACCESSION NUMBER: 2003-15611 BIOTECHDS  
TITLE: Novel DNA with promoter activity, applicable in screening substances regulating expression of sphingosine kinase I as remedies or preventives for e.g. arteriosclerosis, diabetes, thrombosis, inflammation and immunopathy;  
involving recombinant vector plasmid pGV-mediated firefly luciferase gene transfer and expression in Escherichia coli  
AUTHOR: KOHAMA T; SUGIURA M  
PATENT ASSIGNEE: SANKYO CO LTD  
PATENT INFO: WO 2003035871 1 May 2003  
APPLICATION INFO: WO 2002-JP10882 21 Oct 2002  
PRIORITY INFO: JP 2001-325402 23 Oct 2001; JP 2001-325402 23 Oct 2001  
DOCUMENT TYPE: Patent  
LANGUAGE: Japanese  
OTHER SOURCE: WPI: 2003-403346 [38]

AB DERWENT ABSTRACT:  
NOVELTY - DNA sequence comprising a nucleotide sequence from nucleotide-1 at the 5'-terminal to nucleotide-2016 at the 3'-terminal in the nucleotide sequence of (I) with 2075 base pairs; or a DNA hybridizable with a DNA containing a nucleotide sequence complementary with the nucleotide sequence of the DNA above under stringent conditions and

having promoter activity in mammalian cells.

DETAILED DESCRIPTION - DNA molecule is: (1) a DNA sequence comprising a nucleotide sequence from nucleotide-1 at the 5'-terminal to nucleotide-2016 at the 3'-terminal in the nucleotide sequence of (I) with 2075 base pairs; or (2) a DNA hybridizable with a DNA containing a nucleotide sequence complementary with the nucleotide sequence of the DNA in (1) under stringent conditions and having promoter activity in mammalian cells; and (3) a DNA containing a nucleotide sequence having less than or equal to 95% homology with the DNA in (1) and with promoter activity in mammalian cells. INDEPENDENT CLAIMS are also included for the following: (1) a DNA which is: (i) a DNA containing 2016 base pairs ligated to a nucleotide sequence encoding a firefly luciferase at its 5'-terminal in a recombinant plasmid capable of sustaining a transformant *Escherichia coli* pGV-hspk1p SAN 73101 (FERM BP-7780); (ii) a DNA hybridizable with a DNA containing a nucleotide sequence complementary with the nucleotide sequence of the DNA in (i) under stringent conditions and having promoter activity in mammalian cells; and (iii) a DNA containing a nucleotide sequence having less than or equal to 95% homology with the DNA in (i) and with promoter activity in mammalian cells; (2) another DNA sequence containing a nucleotide sequence with nucleotides 1-2016 in the sequence of (I), optionally having promoting activity in mammalian cells; (3) a vector containing any of the DNAs; (4) a recombinant vector pGV-hspk1p SAN 73101 (FERM BP-7780) capable of sustaining the transformant *E. coli* pGV-hspk1p SAN 73101 (FERM BP-7780); (5) a host cell transformed with any of the vectors; (6) transformant *E. coli* pGV-hspk1p SAN 73101 (FERM BP-7780); (7) screening substances having regulatory effect on the promoter activity of sphingosine kinase I gene, e.g. as remedies or preventives for arteriosclerosis, diabetes, thrombosis, inflammation, immunopathy, allergy, cancer and cancer metastasis, comprising: (i) culturing the vector-transformed host cells, in the presence or absence of a test substance; (ii) detecting the produced amount of a protein encoded by a DNA ligated to the 3'-terminal of the already-specified DNA; and (iii) comparing results for selection, particularly the test substance that can lower the expression dose in the cultured cells; (8) screening a cDNA that encodes a protein applicable for the treatment or prevention of arteriosclerosis, diabetes, thrombosis, inflammation, immunopathy, allergy, cancer and cancer metastasis comprising: (i) culturing (A) cells transformed with a vector for transient or stable expression of the test cDNA encoded protein after recombination into a plasmid for expression in mammals, and (B) cells transformed with a similar vector but not expressing the test cDNA encoded protein; (ii) measuring the produced amount of a protein encoded by a DNA ligated to the 3'-terminal of the already-specified DNA; (iii) comparing the results, especially with selection of the cDNA causing a reduction in the protein expression dose in cells (A) as compared with that in cells (B); (9) separating substances regulating the promoter activity of sphingosine kinase I gene by contacting a test substance with any of the DNAs, and then purifying the substance binding to such DNA; (10) screening proteins regulating the promoter activity of sphingosine kinase I gene by contacting a sample of the extract of human cell nucleus with any of the DNAs, and then separating the protein binding with such DNA; (11) screening substances regulating the promoter activity of sphingosine kinase I gene comprising: (i) labeling any of the DNAs then preparing its mixtures with an extract of human cell nucleus or/and a test substance; and (ii) electrophoresis of the mixture and detecting the bands including those containing the labeled DNA with comparison of migration degree between the bands; and (12) a nucleic acid or its derivative containing: (i) less than or equal to 10 consecutive nucleotides in the nucleotide sequence from nucleotides 1-2016 in the nucleotide sequence of (I); or (ii) less than or equal to 10 consecutive nucleotides in an antisense of the nucleotide sequence from nucleotides 1-2016 in the sequence of (I), and having regulatory effect on the promoter activity of sphingosine kinase I.

BIOTECHNOLOGY - Preferred Vectors: Such vector contains a protein-encoded DNA ligated to the 3'-terminal of the already-specified DNA. Such protein is particularly luciferase, horseradish peroxidase, alkali phosphatase, beta-galactosidase or green-fluorescent protein,



particularly luciferase. Preferred Host Cells: The host cell is a prokaryote, or eukaryote, or animal cell.

ACTIVITY - Antiarteriosclerotic; Antidiabetic; Anticoagulant; Thrombolytic; Antiinflammatory; Immunomodulator; Antiallergic; Cytostatic. No biological data is given.

MECHANISM OF ACTION - None given in source material.

USE - The promoter DNA is applicable in screening substances regulating expression of sphingosine kinase I as remedies or preventives for e.g. arteriosclerosis, diabetes, thrombosis, inflammation, immunopathy, allergy, cancer and cancer metastasis (all claimed).

EXAMPLE - Based on results of analysis of the known sphingosine kinase I gene, a luciferase-expression plasmid pGV-hspklp was constructed after recombination. A transformant Escherichia coli was then obtained to produce a protein. The promoter activity of the DNA was studied. No other relevant biological data are available. (35 pages)

L11 ANSWER 15 OF 47 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2003:796515 HCAPLUS

DOCUMENT NUMBER: 139:303797

TITLE: Variants of mammalian sphingosine kinase with reduced catalytic activity and their use in controlling sphingosine-1-phosphate activated processes

INVENTOR(S): Pitson, Stuart M.; Xia, Pu; Moretti, Paul A.; Verwey, Julia R.; Vadas, Mathew A.; Wattenberg, Brian W.

PATENT ASSIGNEE(S): Medvet Science Pty. Ltd., Australia

SOURCE: PCT Int. Appl., 95 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003082322	A1	20031009	WO 2003-AU388	20030328
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW			
RW:	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG			
CA 2480661	AA	20031009	CA 2003-2480661	20030328
EP 1499343	A1	20050126	EP 2003-745226	20030328
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK			
JP 2005526809	T2	20050908	JP 2003-579859	20030328
PRIORITY APPLN. INFO.:			AU 2002-1448	A 20020328
			AU 2002-1538	A 20020405
			AU 2002-1621	A 20020408
			AU 2002-951668	A 20020919
			AU 2003-900230	A 20030121
			WO 2003-AU388	W 20030328

AB The present invention relates generally to a method of modulating cellular activity by modulating the activity of sphingosine kinase by modulating phosphorylation of the enzyme. Modulating phosphorylation of the enzyme modulates the activity of the enzyme and its ability to catalyze formation of the signaling mol. sphingosine-1-phosphate. The present invention still further extends to sphingosine kinase variants and to functional derivs., homologues or analogs, chemical equivalent and mimetics thereof exhibiting reduced and/or ablated capacity to undergo phosphorylation. The method and mols. of the present invention are useful, inter alia, in

the treatment and/or prophylaxis of conditions characterized by aberrant, unwanted or otherwise inappropriate cellular and/or sphingosine kinase functional activity. The present invention is further directed to methods for identifying and/or designing agents capable of modulating sphingosine kinase phosphorylation.

REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 16 OF 47 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2003:76920 HCAPLUS

DOCUMENT NUMBER: 138:132230

TITLE: RPK118, a novel human sphingosine kinase-1-binding protein

INVENTOR(S): Nakamura, Shunichi; Okada, Taro

PATENT ASSIGNEE(S): The New Industry Research Organization, Japan

SOURCE: PCT Int. Appl., 60 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003008582	A1	20030130	WO 2002-JP7352	20020719
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				

PRIORITY APPLN. INFO.: JP 2001-220516 A 20010719

AB A novel sphingosine kinase 1-binding protein RPK118, isolated from human brain, its encoding cDNA, and recombinant expression, are disclosed. Probes and antibodies are claimed. Sphingosine kinase (SPHK) is a key enzyme catalyzing the formation of sphingosine 1 phosphate (SPP), a lipid messenger that is implicated in the regulation of a wide variety of important cellular events through intracellular as well as extracellular mechanisms. However, the mol. mechanism of the intracellular actions of SPP remains unclear. Here the authors have cloned a novel sphingosine kinase-1 (SPHK1)-binding protein, RPK118, by yeast two-hybrid screening. RPK118 contains several functional domains whose sequences are homologous to other known proteins including the phox homol. domain and pseudokinase 1 and 2 domains and is shown to be a member of an evolutionarily highly conserved gene family. The pseudokinase 2 domain of RPK118 is responsible for SPHK1 binding as judged by yeast two-hybrid screening and immunopptn. studies. RPK118 is also shown to co-localize with SPHK1 on early endosomes in COS7 cells expressing both recombinant proteins. Furthermore, RPK118 specifically binds to phosphatidylinositol 3-phosphate. RPK118 binds to sphingosine kinase 1 in the C-terminal side of the P-kinase domain and transports sphingosine kinase 1 to a specific site in a cell via the PX domain and the ESP domain, thereby serving as a sorting protein. These results strongly suggest that RPK118 is a novel SPHK1-binding protein that may be involved in transmitting SPP-mediated signaling into the cell.

REFERENCE COUNT: 12 THERE ARE 12 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 17 OF 47 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2003:792555 HCAPLUS

DOCUMENT NUMBER: 139:377212

TITLE: Sphingosine Kinase Type 2 Is a

AUTHOR(S): Putative BH3-only Protein That Induces Apoptosis  
Liu, Hong; Toman, Rachelle E.; Goparaju, Sravan K.;  
Maceyka, Michael; Nava, Victor E.; Sankala, Heidi;  
Payne, Shawn G.; Bektas, Meryem; Ishii, Isao; Chun,  
Jerold; Milstien, Sheldon; Spiegel, Sarah

CORPORATE SOURCE: Medical College of Virginia Campus, Department of  
Biochemistry, Virginia Commonwealth University,  
Richmond, VA, 23298-0614, USA

SOURCE: Journal of Biological Chemistry (2003), 278(41),  
40330-40336  
CODEN: JBCHA3; ISSN: 0021-9258

PUBLISHER: American Society for Biochemistry and Molecular  
Biology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB There are two isoforms of sphingosine kinase (SphK)  
that catalyze the formation of sphingosine 1-phosphate, a potent  
sphingolipid mediator. Whereas SphK1 stimulates growth and survival, here  
we show that SphK2 enhanced apoptosis in diverse cell types and also  
suppressed cellular proliferation. Apoptosis was preceded by cytochrome c  
release and activation of caspase-3. SphK2-induced apoptosis was  
independent of activation of sphingosine 1-phosphate receptors. Sequence  
anal. revealed that SphK2 contains a 9-amino acid motif similar to that  
present in BH3-only proteins, a pro-apoptotic subgroup of the Bcl-2  
family. As with other BH3-only proteins, co-immunopptn. demonstrated that  
SphK2 interacted with Bcl-xL. Moreover, site-directed mutation of  
Leu-219, the conserved leucine residue present in all BH3 domains,  
markedly suppressed SphK2-induced apoptosis. Hence, the apoptotic effect  
of SphK2 might be because of its putative BH3 domain.

REFERENCE COUNT: 47 THERE ARE 47 CITED REFERENCES AVAILABLE FOR THIS  
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 18 OF 47 MEDLINE on STN DUPLICATE 3

ACCESSION NUMBER: 2003426178 MEDLINE

DOCUMENT NUMBER: PubMed ID: 12815058

TITLE: Role of human sphingosine-1-phosphate phosphatase  
1 in the regulation of intra- and extracellular  
sphingosine-1-phosphate levels and cell viability.

AUTHOR: Johnson Korey R; Johnson Kristy Y; Becker Kevin P;  
Bielawski Jacek; Mao Cungui; Obeid Lina M

CORPORATE SOURCE: Department of Medicine, Medical University of South  
Carolina, Charleston, South Carolina 29425, USA.

CONTRACT NUMBER: 1P20RR17677 (NCRR)  
GM62287 (NIGMS)  
HL 07260 (NHLBI)

SOURCE: Journal of biological chemistry, (2003 Sep 5) 278 (36)  
34541-7. Electronic Publication: 2003-06-18.  
Journal code: 2985121R. ISSN: 0021-9258.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200310

ENTRY DATE: Entered STN: 20030912  
Last Updated on STN: 20031008  
Entered Medline: 20031007

AB Sphingosine-1-phosphate (S1P) is a highly bioactive lipid that exerts  
numerous biological effects both intracellularly as a second messenger and  
extracellularly by binding to its G-protein-coupled receptors of the  
endothelial differentiation gene family (S1P receptors-(1-5)).  
Intracellularly, at least two enzymes, sphingosine  
kinase and S1P phosphatase, regulate the activity of S1P by  
governing the phosphorylation status of S1P. To study the regulation of  
S1P levels, we cloned the human isoform of S1P phosphatase 1  
(hSPPase1). The hSPPase1 has 78% homology to the mouse SPPase  
at the amino acid level with 6-8 possible transmembrane domains. Confocal  
microscopy revealed green fluorescent protein-tagged hSPPase1, expressed  
in either MCF7 or HEK293 cells, co-localized to endoplasmic reticulum with  
calreticulin. According to Northern blot analysis, hSPPase1 is expressed

in most tissues, with the strongest levels found in the highly vascular tissues of placenta and kidney. Transient overexpression of hSPPase1 exhibited a 2-fold increase in phosphatase activity against S1P and dihydro-S1P, indicating that the expressed protein was functional. Small interfering RNA (siRNA) knockdown of endogenous hSPPase1 drastically reduced hSPPase1 mRNA levels, as confirmed by reverse transcription PCR, and resulted in an overall 25% reduction of in vitro phosphatase activity in the membrane fractions. Sphingolipid mass measurements in hSPPase1 siRNA knockdown cells revealed a 2-fold increase of S1P levels and concomitant decrease in sphingosine. In vivo labeling of hSPPase1 siRNA-treated cells showed accumulation of S1P within cells, as well as significantly increased secretion of S1P into the media, indicating that hSPPase1 regulates secreted S1P. In addition, siRNA-induced knockdown of hSPPase1 endowed resistance to tumor necrosis factor-alpha and the chemotherapeutic agent daunorubicin. Collectively, these data suggest that regulation of hSPPase1 with the resultant changes in cellular and secreted S1P could have important implications to cell proliferation, angiogenesis, and apoptosis.

L11 ANSWER 19 OF 47 MEDLINE on STN  
 ACCESSION NUMBER: 2003280908 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 12682045  
 TITLE: Sphingosine phosphate lyase expression is essential for normal development in *Caenorhabditis elegans*.  
 AUTHOR: Mendel Jane; Heinecke Karie; Fyrst Henrik; Saba Julie D  
 CORPORATE SOURCE: Children's Hospital Oakland Research Institute, Oakland, California 94609-1673, USA.  
 CONTRACT NUMBER: 1R01CA77528 (NCI)  
 SOURCE: Journal of biological chemistry, (2003 Jun 20) 278 (25) 22341-9. Electronic Publication: 2003-04-07.  
 Journal code: 2985121R. ISSN: 0021-9258.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200308  
 ENTRY DATE: Entered STN: 20030617  
 Last Updated on STN: 20030822  
 Entered Medline: 20030821

AB Sphingolipids are ubiquitous membrane constituents whose metabolites function as signaling molecules in eukaryotic cells. Sphingosine 1-phosphate, a key sphingolipid second messenger, regulates proliferation, motility, invasiveness, and programmed cell death. These effects of sphingosine 1-phosphate and similar phosphorylated sphingoid bases have been observed in organisms as diverse as yeast and humans. Intracellular levels of sphingosine 1-phosphate are tightly regulated by the actions of sphingosine kinase, which is responsible for its synthesis and sphingosine-1-phosphate phosphatase and sphingosine phosphate lyase, the two enzymes responsible for its catabolism. In this study, we describe the cloning of the *Caenorhabditis elegans* sphingosine phosphate lyase gene along with its functional expression in *Saccharomyces cerevisiae*. Promoter analysis indicates tissue-specific and developmental regulation of sphingosine phosphate lyase gene expression. Inhibition of *C. elegans* sphingosine phosphate lyase expression by RNA interference causes accumulation of phosphorylated and unphosphorylated long-chain bases and leads to poor feeding, delayed growth, reproductive abnormalities, and intestinal damage similar to the effects seen with exposure to *Bacillus thuringiensis* toxin. Our results show that sphingosine phosphate lyase is an essential gene in *C. elegans* and suggest that the sphingolipid degradative pathway plays a conserved role in regulating animal development.

L11 ANSWER 20 OF 47 MEDLINE on STN DUPLICATE 4  
 ACCESSION NUMBER: 2003464494 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 12805067  
 TITLE: A novel MyD-1 (SIRP-1alpha) signaling pathway that inhibits LPS-induced TNFalpha production by monocytes.  
 AUTHOR: Smith Rosemary E; Patel Vanshree; Seatter Sandra D; Deehan Maureen R; Brown Marion H; Brooke Gareth P; Goodridge Helen

S; Howard Christopher J; Rigley Kevin P; Harnett William;  
Harnett Margaret M  
CORPORATE SOURCE: Edward Jenner Institute for Vaccine Research, Compton,  
Newbury, Berkshire, United Kingdom.  
SOURCE: Blood, (2003 Oct 1) 102 (7) 2532-40. Electronic  
Publication: 2003-06-12.  
Journal code: 7603509. ISSN: 0006-4971.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals  
ENTRY MONTH: 200310  
ENTRY DATE: Entered STN: 20031008  
Last Updated on STN: 20031030  
Entered Medline: 20031029

AB MyD-1 (CD172) is a member of the family of signal regulatory phosphatase (SIRP) binding proteins, which is expressed on human CD14+ monocytes and dendritic cells. We now show a novel role for MyD-1 in the regulation of the innate immune system by pathogen products such as lipopolysaccharide (LPS), purified protein derivative (PPD), and Zymosan. Specifically, we demonstrate that ligation of MyD-1 on peripheral blood mononuclear cells (PBMCs) inhibits tumor necrosis factor alpha (TNFalpha) secretion but has no effect on other cytokines induced in response to each of these products. In an attempt to understand the molecular mechanisms underlying this surprisingly selective effect we investigated signal transduction pathways coupled to MyD-1. Ligation of the SIRP was found to recruit the tyrosine phosphatase SHP-2 and promote sequential activation of phosphatidylinositol (PI) 3-kinase, phospholipase D, and sphingosine kinase. Inhibition of LPS-induced TNFalpha secretion by MyD-1 appears to be mediated by this pathway, as the PI 3-kinase inhibitor wortmannin restores normal LPS-driven TNFalpha secretion. MyD-1-coupling to this PI 3-kinase-dependent signaling pathway may therefore present a novel target for the development of therapeutic strategies for combating TNFalpha production and consequent inflammatory disease.

L11 ANSWER 21 OF 47 MEDLINE on STN DUPLICATE 5  
ACCESSION NUMBER: 2003007891 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 12411432  
TITLE: Identification and characterization of a novel  
human sphingosine-1-phosphate phosphohydrolase,  
hSPP2.  
AUTHOR: Ogawa Chie; Kihara Akio; Gokoh Maiko; Igarashi Yasuyuki  
CORPORATE SOURCE: Department of Biomembrane and Biofunctional Chemistry,  
Graduate School of Pharmaceutical Sciences, Hokkaido  
University, Kita 12-jo, Nishi 6-choume, Kita-ku, Sapporo  
060-0812, Japan.  
SOURCE: Journal of biological chemistry, (2003 Jan 10) 278 (2)  
1268-72. Electronic Publication: 2002-10-30.  
Journal code: 2985121R. ISSN: 0021-9258.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-AF542512  
ENTRY MONTH: 200303  
ENTRY DATE: Entered STN: 20030107  
Last Updated on STN: 20030308  
Entered Medline: 20030307

AB Sphingosine 1-phosphate (S1P) is a bioactive lipid molecule that acts as both an extracellular signaling mediator and an intracellular second messenger. S1P is synthesized from sphingosine by sphingosine kinase and is degraded either by S1P lyase or by S1P phosphohydrolase. Recently, mammalian S1P phosphohydrolase (SPP1) was identified and shown to constitute a novel lipid phosphohydrolase family, the SPP family. In this study we have identified a second human S1P phosphohydrolase, SPP2, based on sequence homology to human SPP1. SPP2 exhibited high phosphohydrolase activity against S1P and dihydrosphingosine 1-phosphate. The dihydrosphingosine-1-

phosphate phosphohydrolase activity was efficiently inhibited by excess S1P but not by lysophosphatidic acid, phosphatidic acid, or glycerol 3-phosphate, indicating that SPP2 is highly specific to sphingoid base 1-phosphates. Immunofluorescence microscopic analysis demonstrated that SPP2 is localized to the endoplasmic reticulum. Although the enzymatic properties and localization of SPP2 were similar to those of SPP1, the tissue-specific expression pattern of SPP2 was different from that of SPP1. Thus, SPP2 is another member of the SPP family that may play a role in attenuating intracellular S1P signaling.

L11 ANSWER 22 OF 47 MEDLINE on STN DUPLICATE 6  
ACCESSION NUMBER: 2003519363 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 14596938  
TITLE: The immunosuppressant FTY720 is phosphorylated by sphingosine kinase type 2.  
AUTHOR: Paugh Steven W; Payne Shawn G; Barbour Suzanne E; Milstien Sheldon; Spiegel Sarah  
CORPORATE SOURCE: Department of Biochemistry, Virginia Commonwealth University School of Medicine, Richmond, VA 23298, USA.  
CONTRACT NUMBER: AI50094 (NIAID)  
CA61774 (NCI)  
SOURCE: FEBS letters, (2003 Nov 6) 554 (1-2) 189-93.  
Journal code: 0155157. ISSN: 0014-5793.  
PUB. COUNTRY: Netherlands  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200312  
ENTRY DATE: Entered STN: 20031105  
Last Updated on STN: 20031224  
Entered Medline: 20031223

AB The potent immunosuppressive drug FTY720, a sphingosine analog, induces redistribution of lymphocytes from circulation to secondary lymphoid tissues. FTY720 is phosphorylated in vivo and functions as an agonist for four G-protein-coupled sphingosine-1-phosphate receptors. The identity of the kinase that phosphorylates FTY720 is still not known. Here we report that although both sphingosine kinase type 1 (SphK1) and type 2 (SphK2) can phosphorylate FTY720 with low efficiency, SphK2 is much more effective than SphK1. FTY720 inhibited phosphorylation of sphingosine catalyzed by SphK2 to a greater extent than it inhibits SphK1. Thus, SphK2 may be the relevant enzyme that is responsible for in vivo phosphorylation of FTY720.

L11 ANSWER 23 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN  
DUPLICATE 7  
ACCESSION NUMBER: 2002-13721 BIOTECHDS  
TITLE: Identifying tumor characteristics in a tissue sample taken from a patient, involves determining the copy number or expression level of genes associated with lipid metabolism, synthesis or action;  
DNA probe immobilization on solid support matrix for DNA microarray construction and cancer diagnosis  
AUTHOR: SKINNER M K; PATTON J L; CHAUDHARY J  
PATENT ASSIGNEE: ATAIRGIN TECHNOLOGIES INC  
PATENT INFO: WO 2002027028 4 Apr 2002  
APPLICATION INFO: WO 2000-US30366 28 Sep 2000  
PRIORITY INFO: US 2000-676052 28 Sep 2000  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
OTHER SOURCE: WPI: 2002-405056 [43]

AB DERWENT ABSTRACT:  
NOVELTY - Identifying tumor characteristics, comprising measuring a copy number or expression level of at least two genes associated with lipid metabolism, synthesis, or action in cells from a patient tissue sample, and comparing the results with a copy number or expression level of the genes in a normal cell, is new.  
DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for an array of nucleic acid polymers immobilized on a solid support, comprising a solid support, at least two different nucleic acid polymers which are

each specific for a different gene associated with lipid metabolism, synthesis or action, where each nucleic acid polymer is located at a predetermined position on the solid support, and the array comprises nucleic acid polymers which are specific for less than 100 genes other than the selected genes.

**BIOTECHNOLOGY - Preferred Method:** The genes associated with lipid metabolism, synthesis, or action are phosphatidylinositol-3-kinase (catalytic, alpha-polypeptide), phospholipase D1 (phosphatidylcholine specific), dihydroxyacetone phosphate acyltransferase, phosphate cytidyltransferase 1 (choline specific, alpha form), phosphate cytidyltransferase 2 (ethanolamine specific), sphingosine kinase, phosphatidic acid phosphatase type 2c, human lysophospholipase homolog, prostate differentiation factor PLAB, phospholipase A2, phospholipase C beta3 (phosphatidylinositol specific), phosphatidylinositol-3-kinase (class2, gamma-polypeptide), choline/ethanolamine phosphotransferase, lysophospholipase, aldehyde dehydrogenase (5 family member A1), phospholipase D1 glycosylphosphatidylinositol specific 1-acylglycerol-3-phosphate acyltransferase, phosphatidic acid phosphate type 2a, 2b, EDG1-EDG7, glycerol-3-phosphate dehydrogenase, sphingosine-1-phosphate lyase 1, phosphatase and tension homolog (PTEN), sphingomyelin phosphodiesterase 1, N-acylsphingosine amidohydrolase, glycerol kinase, diacylglycerol kinase gamma, glycerol-3-phosphate acyltransferase, triacylglycerol lipase, phosphatidylserine decarboxylase, CDP diacylglycerol inositol-3-phosphatidyltransferase, sphingosine kinase type 2, mitogen activator protein (MAP) kinase 1, LYPLA2, MAP kinase phosphatase like, lysophosphatidic acid (LPA) phosphatase, phospholipid scramblase 4, protein kinase B and lipid activated protein kinase, interleukin-6 (IL6), IL8, ROCK1, urokinase-type plasminogen activator (uPA), tPA, gro1, vascular endothelial growth factor (VEGF) and heparin-binding epidermal growth factor (hbEGF). The method further comprises measuring a copy number or expression of at least two lipid influenced genes in cells from a patient tissue sample and comparing the results with a copy number of genes in a normal cell, where the lipid influenced genes are ERK1, JNK1, actin B, leutinizing hormone (LH) receptor, follicle stimulating hormone (FSH) receptor, LRP, SPARC, Ras inhibitor (RIN1), insulin-like growth factor (IGF) binding protein 4, SERPINA 8, TIMP1, PIP5K2B, secretory leukocyte protease inhibitor, FLJ20258, Id4, GNA14, SYK, MSP2K4, G protein coupled receptor 39, SIR2, prostate epithelium specific Ets transcription factor, cathepsin D, gonadotropin releasing hormone (GnRH) receptor, profilin, CA 125, CDKN1A, RAB13, FCGRT, RPS9, LATS1, JunD, FGFR4, p66shc, Id3, human polo like kinase, MDM2, Hras, CTNNB1, CDKN1B, AKT2, MGMT, actin bundling protein, MMP7, MMP2, mesothelin, MUC5AC and MAS1. Copy number of the genes is determined by isolating sample nucleic acid polymers from cells of the patient tissue sample, hybridizing the sample nucleic acid polymers with nucleic acid polymers specific for the selected genes to quantify the extent of hybridization, and comparing the hybridization data obtained with data obtained from the hybridization of reference nucleic acid polymers isolated from a normal cell of the same tissue type as the patient tissue sample. The sample nucleic acid polymer is amplified in the isolating step. Hybridization of the sample nucleic acid polymers uses a nucleic acid polymer comprising at least 19 nucleotides to hybridize to a coding region of one of the selected genes, or to a non-coding sequence functionally linked to the coding region of one of the selected genes, where the non-coding functionally linked gene is unique to that gene. The nucleic acid polymers specific for the selected genes are immobilized on a solid support, and each polymer specific for each gene is located at a predetermined position on the solid support.

**USE** - The method is useful for determining tumor characteristics in a tissue sample taken from a patient (claimed).

**EXAMPLE** - cDNA-containing clones corresponding to particular genes was purchased from a commercial source. The cDNA sequence was directly isolated from a sufficient number of insert-containing plasmids and amplified. After confirming the identity of each clone by restriction enzyme digestion, and/or sequencing, the cDNA was amplified with a combination of two standard primers. The amplified cDNA was then gel purified and resuspended in water for spotting onto the derivatized glass slide for the array. The probes were affixed to a glass slide using

the aminosilane linkage chemistry described in Cheung, et al., Making and Reading Microarrays Nature Genetics Supp., 21:15-19 (1999). The probes were arrayed in an asymmetrical pattern. Approximately 10 ng of each probe DNA was deposited in spots 125 micrometers in diameter and 300 micrometers apart. Genomic DNA was extracted from 300 mg of sample tissue, and digested with restriction enzymes. The DNA was labeled with fluorescent dyes, radiolabels, or enzymes with either random priming, nick translation, or end labeling techniques. 40 microg of sample was then amplified by polymerase chain reaction (PCR) with a mixture of fluorescein labeled random hexamer primers using Taq polymerase. The amplified sample DNA was separated from the labeled primers. Purified sample DNA was then resuspended in 15 microliters of hybridization solution (50% formamide, 6XSSC (standard saline citrate), 0.5% sodium dodecyl sulfate (SDS), 5XDenhard's reagent). The microarray was pre-hybridized with hybridization solution for about 30 minutes. The sample DNA were denatured for 4 minutes at 80 degrees C in a hybridization oven. The amplified sample DNA in the hybridization solution was then applied to the microarray in a Corning CMT hybridization chamber, and then allowed to anneal at 42degreesC for 20 hours. After hybridization had been completed, the array slide was washed for 5 minutes in 0.1% SDS/0.2XSSC, and then 5 minutes with 0.2XSSC. The array was then loaded into an Affymetrix 418 Scanner (Affymetrix), and fluorescence of the hybridized sample DNA on the microarray was detected. The correlation between lipid-associated gene copy number and certain tumor characteristics was demonstrated by determining the copy number of lipid-associated genes utilizing a lipid-associated gene probe array. Tumor tissue samples from stage 1, 2, 3 and 4 tumors were assayed, as well as and those characterized as serous, mucinous, endometrioid, low stage and high stage. Tumors from various types of cancers, including ovarian, breast, cervical, and uterine were analyzed, while normal tissues of each type provided a control and a standard curve. This analysis demonstrated that specific tumor stages and characteristics correlated to increases or decreases in the copy number of certain lipid-associated genes. (113 pages)

L11 ANSWER 24 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN  
ACCESSION NUMBER: 2003-01644 BIOTECHDS

TITLE: Inducing blood vessel formation, or preventing/treating  
congestive heart failure, ischemia-reperfusion injury,  
myocardial ischemia and peripheral arterial diseases in  
animal, by administering sphingosine kinase

adeno virus or lenti virus vector-mediated gene transfer  
and expression in mammal cell for cardiovascular disease  
gene therapy

AUTHOR: LIAU G; STEFANSSON S; SU J  
PATENT ASSIGNEE: NOVARTIS AG; NOVARTIS-ERFINDUNGEN VERW GES MBH  
PATENT INFO: WO 2002028406 11 Apr 2002  
APPLICATION INFO: WO 2001-EP11513 5 Oct 2001  
PRIORITY INFO: US 2000-238230 5 Oct 2000; US 2000-238230 5 Oct 2000  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
OTHER SOURCE: WPI: 2002-608171 [65]

AB DERWENT ABSTRACT:

NOVELTY - Inducing blood vessel formation in an animal, or preventing or treating congestive heart failure, myocardial ischemia, ischemia-reperfusion injury and peripheral arterial diseases in an animal, involves administering sphingosine kinase (I), its analog, fragment or derivative, to the animal.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a viral vector (III) including a polynucleotide (II) encoding (I); and (2) expressing (I) in an animal, by administering (II) to the animal.

BIOTECHNOLOGY - Preferred Method: (I) is administered to the animal by administering (II) or an expression vehicle including (II) to the animal. Preferred Vector: The expression vehicle further includes a polynucleotide encoding a protein selected from vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), insulin like growth factor (IGF), angiopoietins, platelet-derived epidermal growth factor



(PD-EGF), tumor growth factor-beta (TGF-beta), hypoxia-inducible factor 1 alpha (HIF1-alpha), nitric oxide synthase, monocyte chemoattractant protein-1 (MCP-1), interleukin 8 (IL-8), ephrins, NAP-2 (undefined), ENA-78 (undefined), GROW-alpha (undefined) or active fragments of tyrosyl-tRNA synthetase. (III) is a viral vector, e.g. adenoviral, lentiviral or BIV (bovine immunodeficiency virus).

ACTIVITY - Cardiant; Vasotropic; Vulnerary; Antiulcer.

MECHANISM OF ACTION - Inducer of blood vessel formation (angiogenesis) (claimed). To determine if Av3sphklalpha induces angiogenesis in vivo, a matrigel implant model in athymic mice was used. S8 cells transduced with Av3sphklalpha (a viral vector encoding mouse sphingosine kinase) at 100 particles per cell were mixed with 0.5 Matrigel and implanted subcutaneously for 7 days. Av3sphklalpha clearly enhanced bovine fibroblast growth factor (bFGF)-induced angiogenesis as demonstrated by histological analysis. Appearance of larger, mature vessels as well as increased number of CD31-positive vessel structure were apparent in the presence of Av3sphklalpha transduced cells. By contrast, the control Av3null vector did not enhance angiogenesis.

USE - The method is useful for inducing blood vessel formation in an animal, or preventing or treating congestive heart failure, myocardial ischemia, ischemia-reperfusion injury and peripheral arterial diseases in an animal, e.g. mammal (such as primate including human) (claimed). (III) is useful for treating diseases or disorders selected from coronary artery disease, peripheral vascular disease, wound healing and fracture repair, reconstructive surgery, transplantation such as islet transplants, tendon repair/sports injury, healing of ulcers, thromboangitis obliterans (Buerger's disease), periodontal tissue regeneration and radiotherapy-induced esophagitis.

ADMINISTRATION - The adenoviral vector is administered at a dose of 107-1012, preferably 5 x 108- 2 x 1011 plaque forming units (pfu). Lentivirus vector is administered at a dose of 5 x 105-1012, preferably 5 x 105-1010 transducing units (claimed). Administration route for the vectors is not given in the specification.

EXAMPLE - Plasmid pCR3.1sphKlalpha, derived from pCR3.1, contained mouse sphingosine kinase alpha cDNA. pCR3.1sphKlalpha was digested with HindIII and NotI to isolate a 1531 bp insert containing the coding sequence for sphKlalpha. The fragment was blunt-ended and cloned into EcoRV site of pAVS6alx, an adenoviral shuttle plasmid containing a lox recombination site, to create pAV1xsphKlalpha, pAVS61alx was formed by adding a lox site to pAVS6a. A 535 bp ClaI/NcoI fragment from pAVH8-101 1x, containing the SV40 polyA signal and lox site was inserted into pAVS6a digested with ClaI and NeoI and linearized (4745 bp). The sphKlalpha cDNA was cloned downstream of the RSV promoter, and the adenoviral tripartite leader sequence, and included the SV40 polyadenylation signal and a homologous recombination region. A large-scale plasmid preparation was prepared using the alkaline lysis method and purified using a CsTFA gradient following standard protocols. The cDNA was then sequenced. The sphKlalpha coding sequence was 1149 kb and encoded a 382 amino acid protein, fully defined in the specification. The sphKlalpha cDNA was incorporated into an adenoviral vector using the lox recombination three-plasmid transfection system. AE1-2a (also called as S8 cells) cells were cultured in Richters media containing 5% heat inactivated fetal bovine serum (FBS). Transient transfections of the AE1-2a cells were performed with the micrograms of NotI-digested pAV1xsphklalpha, 0.5 micrograms of pCcre and 1 microgram of ClaI-digested pSQ3 DNA using Lipofectamine-PLUS reagent system. Plasmid pSQ3 was a 31574 bp plasmid containing adenoviral structural genes, but was devoid of E1, E2a and E3 sequences. S8 cells were cultured in Lipofectamine reagent/DNA precipitate at 37 degrees Centigrade for 16 hours, and then in Richters media for 5-7 days. A cytopathic effect was observed in cells approximately after 12-15 days post- transfection. The virus was amplified in 15 cm dishes of dexamethasone-induced S8 cells. The recombinant Av3sphklalpha vector was purified and a large scale seedlot was prepared. (38 pages)

exhibit reduced catalytic activity useful for modulating cellular functional activity and treating or preventing inflammatory, degenerative diseases and neoplastic conditions

mutant **sphingosine-kinase** produced by site-directed mutagenesis useful for gene therapy and prophylaxis

AUTHOR: PITSON S; MORETTI P; ZEBOL J; XIA P; GAMBLE J; VADAS M; D'ANDREA R; WATTENBERG B  
PATENT ASSIGNEE: MEDVET SCI PTY LTD  
PATENT INFO: WO 2002000887 3 Jan 2002  
APPLICATION INFO: WO 2000-AU730 28 Jun 2000  
PRIORITY INFO: AU 2001-2749 29 Jan 2001  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
OTHER SOURCE: WPI: 2002-130896 [17]

AB DERWENT ABSTRACT:

NOVELTY - A **sphingosine kinase** variant (I), comprising a mutation in a **sphingosine kinase** binding region defined by amino acids 16-153 or a ATP binding site region (or their functionally equivalent regions), where the variant exhibits ablated or reduced catalytic activity relative to wild-type SK, or a derivative, **homolog**, analog, chemical equivalent or mimetic of the SK variant, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated nucleic acid molecule (II), its derivative or equivalent, comprising a nucleotide sequence encoding or complementary to a sequence encoding (I); (2) detecting an agent capable of modulating the interaction of FOSK (friends of SK) with SK or its functional equivalent or derivative, by contacting a cell or its extract containing the SK or FOSK or its functional equivalent or derivative with a putative agent and detecting an altered expression phenotype associated with the interaction; (3) detecting an agent capable of binding or otherwise associating with the SK region defined by amino acid 16-153 (or its functional equivalent or derivative), by contacting a cell containing the amino acid region with a putative agent and detecting an altered expression phenotype associated with modulation of the function of SK; (4) analyzing, designing and/or modifying an agent capable of interacting with SK region defined by amino acid 16-153 or its derivative and modulating a functional activity associated with SK, by contacting SK or its derivative with a putative agent and assessing the degree of interactive complementarity of the agent with the binding site; (5) an agent (II) identified by the method of (2), (3) or (4); and (6) a pharmaceutical composition comprising (I) or (II).

BIOTECHNOLOGY - Preparation: (I) is derived from natural or recombinant sources. Preferred Variant: SK is **human** SK and comprises a single or multiple amino acid substitution, addition and/or deletion. The SK binding region is defined by amino acids 70-90, preferably 79-84. The variant exhibits ablated catalytic activity, particularly a reduced capacity to phosphorylate sphingosine to sphingosine 1-phosphate.

ACTIVITY - Antiinflammatory; Antirheumatic; Antiarthritic; Cytostatic; Antiasthmatic; Antiatherosclerotic; Neuroprotective; Antibacterial; Immunosuppressive; Osteopathic. No biological data is given.

MECHANISM OF ACTION - Modulator of SK/FOSK interactivity; Inhibitor of wild-type SK activation; Regulator of cellular functional activity including chemokine, cytokine and inflammatory modulator production; gene therapy.

USE - (I) and (II) are useful for modulating cellular functional activity, down-regulating wild-type SK baseline activity and/or preventing wild-type SK activation. (I) and (II) are also useful for treatment and/or prophylaxis of a condition in a mammal, characterized by aberrant, unwanted or inappropriate cellular activity, and in the manufacture of a medicament for modulating cellular functional activity. (All claimed). (I) is useful in therapeutically or prophylactically treating inflammatory diseases (e.g. rheumatoid arthritis, inflammatory bowel disease), neoplastic conditions (e.g. solid cancer), asthma, atherosclerosis, meningitis, multiple sclerosis, septic shock,

osteoarthritis, and other degenerative diseases.

ADMINISTRATION - Administered by oral, intravenous, intranasal, intraperitoneal, intramuscular, subcutaneous, intradermal or suppository route at a dose of 0.1-1 mg/kg/day.

EXAMPLE - The sphingosine kinase (SK-1) cDNA Pitson et al., 2000 was cloned into pALTER site directed mutagenesis vector. Single-stranded DNA was prepared and used as template for oligonucleotide directed mutagenesis. The mutagenic oligonucleotide (5'-CTGGAGACGATCTGATGCAC) was designed to generate the G82D mutant, substitution of the glycine at position 82 to aspartic acid. The mutagenic oligonucleotide (5'-GTCTGGAGATGCATTGATGCACG-3') was designed to generate the SK(G82A) mutant, substitution of the glycine at position 82 to alanine. The mutants were sequenced to verify incorporation of the desired modification and sub-cloned into pcDNA3. The expression construct was transfected by calcium phosphate precipitation into HEK293T cells. The G82DSK by itself had no SK activity and did not suppress endogenous baseline SK activity, however it totally suppressed the increases in SK activity seen after treatment of cells with activating agents such as tumor necrosis factor (TNF), interleukin-1 (IL-1) and PMA (phorbol-12-myristate-13-acetate). G82DSK inhibited SK stimulated by the oncogene Ras and suppressed in vitro and in vivo markers of oncogenesis. The inhibitor was specific as it didn't depress the activation of another enzyme protein kinase C or sphingomyelinase. Human SK(G82A) had catalytic activity much lower than the wild-type hSK. Analysis of the substrate kinetics of hSK(G82A) showed that this mutant had considerably lower affinity for ATP than the wild-type hSK, while the affinity for sphingosine remained unaffected. The kinetic data indicated that Gly82 was involved in ATP binding and this residue was a part of the ATP-binding site of HSK. (104 pages)

L11 ANSWER 26 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

ACCESSION NUMBER: 2002-17812 BIOTECHDS

TITLE: New isolated sphingosine kinase, useful in identifying modulators for treating e.g. cancer, also related nucleic acid, vectors and transformed cells; vector-mediated gene transfer, expression in host cell and antibody for recombinant protein production, drug screening and disease therapy

AUTHOR: SPIEGEL S  
PATENT ASSIGNEE: SPIEGEL S  
PATENT INFO: US 2002042358 11 Apr 2002  
APPLICATION INFO: US 2000-796487 2 Mar 2000  
PRIORITY INFO: US 2001-796487 2 Mar 2001  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
OTHER SOURCE: WPI: 2002-478846 [51]

AB DERWENT ABSTRACT:  
NOVELTY - Isolated sphingosine kinase (SPHK) DNA (I) and parts of it.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) cells transfected with (I); (2) testing compounds (M1) for their effect on SPHK activity; (3) recombinant DNA comprising (I) and a vector; (4) preparation (M2) of SPHK peptides (II) by culturing cells of (1); (5) isolated (II) produced by M2; (6) screening (M3) for compounds that reduce, eliminate or promote SPHK activity; (7) detecting (M4) SPHK by reaction with antibodies (Ab); (8) agents (A) that inhibit or promote SPHK activity; (9) detecting (M5) SPHK by polymerase chain reaction; (10) diagnostic kit for detecting SPHK RNA/cDNA comprising primers or oligonucleotides; (11) measuring (M6) sphingosine-1-phosphate (SPP); (12) decreasing (M7) cell proliferation by reducing SPHK activity or expression; and (13) reducing (M8) cell death by increasing SPHK activity or expression.

WIDER DISCLOSURE - Also disclosed are antibodies (Ab) specific for SPHK.

BIOTECHNOLOGY - Preferred Nucleic Acid: (I) is (i) human or (ii) murine, and then comprises the sequences of genbank AF068748 or 068749, or their fragments of at least 30 nucleotides. Particularly (I) encodes a 381 amino acid SPHK, or its natural or synthetic variants, or fragment of at least 10 amino acids. (I) may be incorporated into

prokaryotic or eukaryotic vectors. Preparation: A 49 kD SPHK was isolated from rat kidney and the sequences of several tryptic peptides determined. These were used in homology searching to identify two murine sequences of 388 and 381 amino acids (mSPHK1a and 1b). The sequence for humanSPHK1 was obtained by reverse transcription polymerase chain reaction on RNA from HEK293 cells and a gene-specific antisense primer. The cDNA formed was extended by the rapid amplification of cDNA ends process and the complete sequence has been deposited as AF238083. Once isolated, the human cDNA can be cloned into e.g. pcDNA3.1 or pCR3.1 for expression in HEK293 cells. Preferred Method: In M1, a test compound is incubated with (i) control cells and (ii) cells transformed to express (I) so that sphingosine metabolites (SM) are formed, and the levels of SM and SPHK are measured and compared. In M3, cells transfected with the vector of (3) are incubated with test compound and any change in SPHK-dependent phosphorylation of lipids, relative to controls, is measured. In M1, SPP is isolated from other phospholipids in a sample, converted to sphingosine, and this phosphorylated using a detectably labeled phosphate. The amount of label that becomes incorporated is then measured.

ACTIVITY - Cytostatic; vasotropic; antidiabetic; neuroprotective. No supporting data is given.

MECHANISM OF ACTION - Modulating production of sphingosine-1-phosphate, a regulator of mitogenesis, apoptosis, atherosclerosis and inflammatory reactions. No supporting data is given.

USE - Cells transformed with (I) are used to screen for agents that reduce, eliminate or promote SPHK activity. Agents that inhibit activity are useful for decreasing cell proliferation, e.g. for treating cancer, and for treating diseases associated with abnormal migration and motility of cells, e.g. restenosis or diabetic neuropathy. Agents that increase activity are used to reduce cell death (claimed). Antibodies (Ab) raised against SPHK, and primers or oligonucleotides derived from (I) are useful for diagnosis; Ab are also useful as therapeutic inhibitors.

ADMINISTRATION - No administration or dosage details are given.

EXAMPLE - No suitable example is given. (24 pages)

L11 ANSWER 27 OF 47 MEDLINE on STN

ACCESSION NUMBER: 2002731982 MEDLINE

DOCUMENT NUMBER: PubMed ID: 12393916

TITLE: The nucleotide-binding site of human sphingosine kinase 1.

AUTHOR: Pitson Stuart M; Moretti Paul A B; Zebol Julia R; Zareie Reza; Derian Claudia K; Darrow Andrew L; Qi Jenson; D'Andrea Richard J; Bagley Christopher J; Vadas Mathew A; Wattenberg Binks W

CORPORATE SOURCE: Hanson Institute, Division of Human Immunology, Institute of Medical and Veterinary Science, Frome Road, Adelaide SA 5000, Australia.. stuart.pitson@imvs.sa.gov.asu

SOURCE: Journal of biological chemistry, (2002 Dec 20) 277 (51) 49545-53. Electronic Publication: 2002-10-18. Journal code: 2985121R. ISSN: 0021-9258.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200302

ENTRY DATE: Entered STN: 20021227

Last Updated on STN: 20030214

Entered Medline: 20030212

AB Sphingosine kinase catalyzes the formation of sphingosine 1-phosphate, a lipid second messenger that has been implicated in a number of agonist-driven cellular responses including mitogenesis, anti-apoptosis, and expression of inflammatory molecules. Despite the importance of sphingosine kinase, very little is known regarding its structure or mechanism of catalysis. Moreover, sphingosine kinase does not contain recognizable catalytic or substrate-binding sites, based on sequence motifs found in other kinases. Here we have elucidated the nucleotide-binding site of human sphingosine kinase 1 (hSK1) through a combination of site-directed mutagenesis and affinity labeling with the

ATP analogue, FSBA. We have shown that Gly(82) of hSK1 is involved in ATP binding since mutation of this residue to alanine resulted in an enzyme with an approximately 45-fold higher K(m)((ATP)). We have also shown that Lys(103) is important in catalysis since an alanine substitution of this residue ablates catalytic activity. Furthermore, we have shown that this residue is covalently modified by FSBA. Our data, combined with amino acid sequence comparison, suggest a motif of SGDGX(17-21)K is involved in nucleotide binding in the sphingosine kinases. This motif differs in primary sequence from all previously identified nucleotide-binding sites. It does, however, share some sequence and likely structural similarity with the highly conserved glycine-rich loop, which is known to be involved in anchoring and positioning the nucleotide in the catalytic site of many protein kinases.

L11 ANSWER 28 OF 47 MEDLINE on STN DUPLICATE 8  
ACCESSION NUMBER: 2002448136 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 12077123  
TITLE: Identification and characterization of RPK118, a novel sphingosine kinase-1-binding protein.  
AUTHOR: Hayashi Shun; Okada Taro; Igarashi Nobuaki; Fujita Toshitada; Jahangeer Saleem; Nakamura Shun-Ichi  
CORPORATE SOURCE: Division of Biochemistry, Department of Molecular and Cellular Biology, Kobe University Graduate School of Medicine, Kobe 650-0017, Japan.  
SOURCE: Journal of biological chemistry, (2002 Sep 6) 277 (36) 33319-24. Electronic Publication: 2002-06-20. Journal code: 2985121R. ISSN: 0021-9258.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200210  
ENTRY DATE: Entered STN: 20020904  
Last Updated on STN: 20030105  
Entered Medline: 20021029

AB Sphingosine kinase (SPHK) is a key enzyme catalyzing the formation of sphingosine 1 phosphate (SPP), a lipid messenger that is implicated in the regulation of a wide variety of important cellular events through intracellular as well as extracellular mechanisms. However, the molecular mechanism of the intracellular actions of SPP remains unclear. Here we have cloned a novel sphingosine kinase-1 (SPHK1)-binding protein, RPK118, by yeast two-hybrid screening. RPK118 contains several functional domains whose sequences are homologous to other known proteins including the phox homology domain and pseudokinase 1 and 2 domains and is shown to be a member of an evolutionarily highly conserved gene family. The pseudokinase 2 domain of RPK118 is responsible for SPHK1 binding as judged by yeast two-hybrid screening and immunoprecipitation studies. RPK118 is also shown to co-localize with SPHK1 on early endosomes in COS7 cells expressing both recombinant proteins. Furthermore, RPK118 specifically binds to phosphatidylinositol 3-phosphate. These results strongly suggest that RPK118 is a novel SPHK1-binding protein that may be involved in transmitting SPP-mediated signaling into the cell.

L11 ANSWER 29 OF 47 MEDLINE on STN  
ACCESSION NUMBER: 2002463181 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 12080051  
TITLE: Cloning and characterization of a protein kinase A anchoring protein (AKAP)-related protein that interacts with and regulates sphingosine kinase 1 activity.  
AUTHOR: Lacana Emanuela; Maceyka Michael; Milstien Sheldon; Spiegel Sarah  
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, Georgetown University Medical School, Washington, D. C. 20007, USA.  
CONTRACT NUMBER: R01 CA61774 (NCI)  
SOURCE: Journal of biological chemistry, (2002 Sep 6) 277 (36) 32947-53. Electronic Publication: 2002-06-21.

Journal code: 2985121R. ISSN: 0021-9258.

PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200210  
ENTRY DATE: Entered STN: 20020912  
Last Updated on STN: 20030105  
Entered Medline: 20021029

AB Sphingosine-1-phosphate (S1P) is a bioactive sphingolipid metabolite that has novel dual actions. S1P is the ligand for a family of G protein-coupled receptors known as S1PRs that mediate various physiological functions. Growth factors rapidly activate sphingosine kinase type 1 (SPHK1) resulting in phosphorylation of sphingosine to form S1P, which plays important roles in cell growth regulation and protection from apoptosis. However, little is known of the mechanism(s) by which SPHK activity is regulated. Using a yeast two-hybrid screening approach, we cloned a 3-kb cDNA encoding a SPHK1-interacting protein (SKIP). BLAST analysis revealed that SKIP corresponded to the C-terminal region of a larger (approximately 7 kb) cDNA that encoded a protein with a high degree of similarity to a family of protein kinase A anchor proteins (AKAP). In confirmation of the yeast two-hybrid assay, glutathione S-transferase (GST)-SPHK1 specifically pulled down SKIP, whereas GST did not. Moreover, immunoprecipitation of in vitro translated SPHK1 and SKIP revealed that SKIP and SPHK1 are tightly associated. Furthermore, SKIP overexpression in NIH 3T3 fibroblasts reduced SPHK1 activity and interfered with its biological functions. The apoptotic-sparing effect of SPHK1 against serum deprivation was reduced when co-transfected with SKIP. In addition, SPHK1-enhanced cell proliferation was also abolished by SKIP, with a corresponding decrease in activation of ERK. Taken together, these results indicate that SKIP is a novel protein likely to play a regulatory role in the modulation of SPHK1 activity.

L11 ANSWER 30 OF 47 MEDLINE on STN DUPLICATE 9

ACCESSION NUMBER: 2002347257 MEDLINE

DOCUMENT NUMBER: PubMed ID: 11956206

TITLE: Ceramide kinase, a novel lipid kinase. Molecular cloning and functional characterization.

AUTHOR: Sugiura Masako; Kono Keita; Liu Hong; Shimizugawa Tetsuya; Minekura Hiroyuki; Spiegel Sarah; Kohama Takafumi

CORPORATE SOURCE: Pharmacology and Molecular Biology Research Laboratories, Sankyo Co., Ltd., Tokyo 140-8710, Japan.

SOURCE: Journal of biological chemistry, (2002 Jun 28) 277 (26) 23294-300. Electronic Publication: 2002-04-15.  
Journal code: 2985121R. ISSN: 0021-9258.

PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-AB079066; GENBANK-AB079067  
ENTRY MONTH: 200208  
ENTRY DATE: Entered STN: 20020702  
Last Updated on STN: 20030105  
Entered Medline: 20020806

AB Ceramide-1-phosphate is a sphingolipid metabolite that has been implicated in membrane fusion of brain synaptic vesicles and neutrophil phagolysosome formation. Ceramide-1-phosphate can be produced by ATP-dependent ceramide kinase activity, although little is known of this enzyme because it has not yet been highly purified or cloned. Based on sequence homology to sphingosine kinase type 1, we have now cloned a related lipid kinase, human ceramide kinase (hCERK). hCERK encodes a protein of 537 amino acids that has a catalytic region with a high degree of similarity to the diacylglycerol kinase catalytic domain. hCERK also has a putative N-myristoylation site on its NH(2) terminus followed by a pleckstrin homology domain. Membrane but not cytosolic fractions from HEK293 cells transiently transfected with a hCERK expression vector readily phosphorylated ceramide but not sphingosine or other sphingoid bases, diacylglycerol or

phosphatidylinositol. This activity was clearly distinguished from those of bacterial or human diacylglycerol kinases. With natural ceramide as a substrate, the enzyme had a pH optimum of 6.0-7.5 and showed Michaelis-Menten kinetics, with K(m) values of 187 and 32 microm for ceramide and ATP, respectively. Northern blot analysis revealed that hCERK mRNA expression was high in the brain, heart, skeletal muscle, kidney, and liver. A BLAST search analysis using the hCERK sequence revealed that putative ceramide kinases (CERKs) exist widely in diverse multicellular organisms including plants, nematodes, insects, and vertebrates. Phylogenetic analysis revealed that CERKs are a new class of lipid kinases that are clearly distinct from sphingosine and diacylglycerol kinases. Cloning of CERK should provide new molecular tools to investigate the physiological functions of ceramide-1-phosphate.

L11 ANSWER 31 OF 47 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2002:126894 HCAPLUS

DOCUMENT NUMBER: 136:322993

TITLE: Relationships and differentially expressed genes among pancreatic cancers examined by large-scale serial analysis of gene expression

AUTHOR(S): Ryu, Byungwoo; Jones, Jessa; Blades, Natalie J.; Parmigiani, Giovanni; Hollingsworth, Michael A.; Hruban, Ralph H.; Kern, Scott E.

CORPORATE SOURCE: Department of Oncology, The Johns Hopkins Medical Institutions, Baltimore, MD, 21231, USA

SOURCE: Cancer Research (2002), 62(3), 819-826

CODEN: CNREA8; ISSN: 0008-5472

PUBLISHER: American Association for Cancer Research

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Pancreatic adenocarcinoma is among the most fatal of cancers, in part because of late diagnosis and a lack of effective therapies. Comprehensive studies are needed to better understand and address the cellular mechanisms and pathways of tumorigenesis. Serial anal. of gene expression was used to analyze gene expression profiles of pancreatic cancer cell lines, short-term cultures of normal pancreatic ductal epithelium, and primary pancreatic cancer tissue. A total of 294,920 tags representing 77,746 genes in 10 serial anal. of gene expression libraries were analyzed. A pancreatic cancer cell line (Hs766T) that exhibited a "normoid" profile of gene expression was identified. Several genes that may be involved in the fundamental nature of malignant changes in pancreatic ductal epithelium were suggested from those differentially and highly expressed in pancreatic cancer cells as compared with normal epithelium. Some overexpressed genes, such as S100A4, prostate stem cell antigen, carcinoembryonic antigen-related cell adhesion mol. 6, and mesothelin, suggest potential use as diagnostic markers. Others suggest potential novel therapeutic targets.

REFERENCE COUNT: 37. THERE ARE 37 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 32 OF 47 MEDLINE on STN

ACCESSION NUMBER: 2002359120 MEDLINE

DOCUMENT NUMBER: PubMed ID: 12102559

TITLE: Sphingosine kinases: a novel family of lipid kinases.

AUTHOR: Liu Hong; Chakravarty Debyani; Maceyka Michael; Milstien Sheldon; Spiegel Sarah

CORPORATE SOURCE: Department of Biochemistry, Virginia Commonwealth University, Richmond 23298, USA.

CONTRACT NUMBER: CA61774 (NCI)

GM43880 (NIGMS)

SOURCE: Progress in nucleic acid research and molecular biology, (2002) 71 493-511. Ref: 85

Journal code: 0102753. ISSN: 0079-6603.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

General Review; (REVIEW)

(REVIEW, TUTORIAL)

LANGUAGE: English

FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200301  
ENTRY DATE: Entered STN: 20020710  
Last Updated on STN: 20030130  
Entered Medline: 20030129

AB Sphingosine kinase (SPHK) catalyzes the formation of sphingosine-1-phosphate (S1P). S1P plays an important role in regulation of a variety of biological processes through intracellular and extracellular actions. S1P has recently been shown to be the ligand for the EDG-1 family of G-protein-coupled receptors. To date, seven cloned SPHKs have been reported with confirmed SPHK activity, including human, mouse, yeast, and plant. A computer search of various databases suggests that a new SPHK family is emerging. The cloning and manipulation of SPHK genes will no doubt provide us with important information about the functions of S1P in a wide range of organisms.

L11 ANSWER 33 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

ACCESSION NUMBER: 2002-09980 BIOTECHDS

TITLE: Human ceramide kinase gene and the enzyme encoded by it for screening substances as drugs for neurological, inflammatory and other disorders;  
recombinant enzyme gene, vector expression in host cell, antibody, database and polymerase chain reaction useful in disease gene therapy

AUTHOR: SUGIURA M; KONO K; KOHAMA T

PATENT ASSIGNEE: SANKYO CO LTD

PATENT INFO: WO 2001096575 20 Dec 2001

APPLICATION INFO: WO 2000-JP4889 14 Jun 2000

PRIORITY INFO: JP 2000-178039 14 Jun 2000

DOCUMENT TYPE: Patent

LANGUAGE: Japanese

OTHER SOURCE: WPI: 2002-179513 [23]

AB DERWENT ABSTRACT:

NOVELTY - A ceramide kinase (CERK1) of human origin having a 537 residue amino acid sequence fully defined in the specification, also ceramide kinase enzymes derived from this sequence by addition, deletion and/or substitution of one or more amino acid residues, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) DNA encoding the novel ceramide kinase; (2) expression vectors containing the DNA of (1); (3) host cells transformed by the vectors of (2); (4) preparing the enzyme by culture of the transformed cells of (3); (5) isolation of the enzyme from samples containing it by co-absorption with calmodulin on to an affinity column, and elution from the column by a solvent containing ethylenedis(oxyethylenenitrilo)tetraacetic acid (EGTA) or ethylenediaminetetraacetic acid (EDTA); (6) screening compounds for their ability to inhibit the ceramide kinase by measuring the extent of phosphorylation of a substrate for the enzyme in the presence or absence of the inhibitor; (7) kits for the screening method of (6); (8) antibodies binding to the enzyme; (9) drug compositions containing DNA encoding all or part of the enzyme; (10) oligonucleotides 15-30 bases in length hybridizing to DNA encoding the enzyme; and (11) drug compositions containing the oligonucleotides of (10).

ACTIVITY - Neuroprotective; antiinflammatory; anti-HIV (human immunodeficiency virus); antidiabetic; anorectic; antibacterial; antiarteriosclerotic; cytostatic. No biological data is given.

MECHANISM OF ACTION - ATP-mediated 1-phosphorylation of ceramides.

USE - For treatment and prevention of disorders including neurological disease, inflammation, human immunodeficiency virus (HIV) infection, diabetes (type 2), obesity, sepsis, arteriosclerosis and cancer.

EXAMPLE - The NCBI expressed sequence tag (EST) database is searched using the tblastn algorithm for homologies to mouse sphingosine kinase 1. A tag (GenBank AA355581) is identified and primes synthesized enclosing it. These are used for polymerase chain reaction amplification of a cDNA library from human leukemia cells (Clontech). The amplification fragment is used to screen a colonic cDNA library in lambda ZAP-II phage. Clones of 1.5 kbase, 3.5 kbase and 4.4 kbase are isolated and sequenced. Together



these encode the sequence of a new ceramide kinase (CERK1). This is inserted into pCR3.1 vector (Invitrogen) and used to transform HEK293 cells (ATCC CRL-1573). The phosphorylation activity of a culture of these cells using N-hexanoyl-D-erythro-sphingosine as substrate is 1280 pmol/minute/mg compared to 7 pmol/minute/mg for cells transformed by pCR3.1 vector only. (61 pages)

L11 ANSWER 34 OF 47 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2001:320122 HCAPLUS  
DOCUMENT NUMBER: 134:337616  
TITLE: Human sphingosine kinase gene  
INVENTOR(S): Allen, Janet; Gosink, Mark; Melendez, Alirio J.; Takacs, Laszlo  
PATENT ASSIGNEE(S): Warner-Lambert Co., USA  
SOURCE: PCT Int. Appl., 91 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001031029	A2	20010503	WO 2000-EP9498	20001027
WO 2001031029	A3	20020228		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
CA 2389127	AA	20010503	CA 2000-2389127	20001027
BR 2000015138	A	20020716	BR 2000-15138	20001027
EP 1228221	A2	20020807	EP 2000-971299	20001027
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL				
JP 2003512072	T2	20030402	JP 2001-533164	20001027
PRIORITY APPLN. INFO.:				
			US 1999-162307P	P 19991028
			US 2000-180525P	P 20000207
			WO 2000-EP9498	W 20001027

AB The present invention relates to the human sphingosine kinase type 1 gene. More precisely the invention concerns a purified or isolated nucleic acid of said sphingosine kinase or a sequence complementary thereto, or fragments thereof. The invention includes oligonucleotides probes or primers for DNA amplification and detection, recombinant polypeptides, recombinant vectors, host cells, as well as antibody production, methods of screening inhibitors, antisense oligonucleotide, transgenic or gene knockout mammals. Sphingosine-1-phosphate (SPP), the product of sphingosine kinase, is an important signaling mol. with intra- and extracellular functions. The cDNA for the mouse sphingosine kinase has recently been reported. In this paper we describe the cloning, expression and characterization of the human sphingosine kinase (huSPHK1). Sequence anal. comparison revealed that this kinase is evolutionarily very conserved, having a high degree of homol. with the murine enzyme, and presenting several conserved regions with bacteria, yeast, plant, and mammalian proteins. Expressed huSPHK1 cDNA specifically phosphorylates D-erythro-sphingosine and, to a lesser extent, D,L-erythro-dihydrosphingosine, and not at all the 'threo' isoforms of dihydrosphingosine; hydroxy-ceramide or non-hydroxy-ceramide; diacylglycerol (DAG); phosphatidylinositol (PI); phosphatidylinositol-4-phosphate (PIP); or phosphatidylinositol-4,5-bisphosphate (PIP2). HuSPHK1 shows typical Michaelis-Menten kinetics ( $V_{max}=56 \mu M$  and  $K_m=5 \mu M$ ). The kinase is inhibited by D,L-threo-dihydrosphingosine ( $K_i=3 \mu M$ ), and

by N,N-dimethyl-sphingosine ( $K_i=5 \mu\text{M}$ ). Northern blots indicate highest expression in adult lung and spleen, followed by peripheral blood leukocyte, thymus and kidney, resp. It is also expressed in brain and heart. In addition, database searches with the stSG2854 sequence indicate that huSPHK1 is also expressed in endothelial cells, retinal pigment epithelium, and senescent fibroblasts.

L11 ANSWER 35 OF 47 MEDLINE on STN DUPLICATE 10  
ACCESSION NUMBER: 2001286242 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 11284453  
TITLE: An improved high-performance liquid chromatographic method for the determination of sphingosine-1-phosphate in complex biological materials.  
AUTHOR: Ruwisch L; Schafer-Korting M; Kleuser B  
CORPORATE SOURCE: Institut für Pharmazie, Pharmakologie und Toxikologie, Freie Universität Berlin, Germany.  
SOURCE: Naunyn-Schmiedeberg's archives of pharmacology, (2001 Mar) 363 (3) 358-63.  
Journal code: 0326264. ISSN: 0028-1298.  
PUB. COUNTRY: Germany: Germany, Federal Republic of  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200105  
ENTRY DATE: Entered STN: 20010529  
Last Updated on STN: 20010529  
Entered Medline: 20010524

AB Sphingosine-1-phosphate (SPP) has been proposed to act both as an intracellular second messenger and as an extracellular mediator via specific cell surface receptors. Based on the increasing diverse cellular roles methods to quantify endogenous and exogenous SPP are highly required. Here, we report a rapid HPLC method that allows quantification of SPP in the picomolar range even in complex biological systems. A two-step lipid extraction serves to separate SPP from most interfering phospholipids and sphingolipids. Importantly, dihydrosphingosine-1-phosphate (dihydro-SPP), not detectable in all cultured cells and biological samples in considerable amounts, possesses equal extraction properties and therefore is an ideal internal standard. Following extraction SPP and dihydro-SPP are converted to fluorescent isoindol derivatives by ortho-phthaldialdehyde (OPA) and separated by HPLC using a gradient program with methanol and 0.07 M  $\text{K}_2\text{HPO}_4$  as eluents. With this procedure we were able to obtain reproducible measurements of SPP over a broad range from 0.5 pM to 0.2 nM. The identity of SPP and dihydro-SPP was confirmed by the use of the ion pair reagent tetraammoniumsulfate, which induced a shift of both peaks but did not alter peak areas. Moreover, enzymatic conversions to sphingosine and sphinganine by bovine intestinal mucosa alkaline phosphatase (AP) excluded the existence of overlapping compounds. Levels of SPP were determined in a variety of biological samples like serum, thrombocytes, primary keratinocytes and several cell lines. Furthermore, we were able to detect increases of intracellular SPP levels in human keratinocytes after exposure to 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> (1,25-(OH)<sub>2</sub>D<sub>3</sub>) for which a stimulation of sphingosine kinase activity has been recognized.

L11 ANSWER 36 OF 47 HCAPLUS COPYRIGHT 2005 ACS on STN  
ACCESSION NUMBER: 2001:187083 HCAPLUS  
DOCUMENT NUMBER: 135:283870  
TITLE: E2Fs regulate the expression of genes involved in differentiation, development, proliferation, and apoptosis  
AUTHOR(S): Muller, Heiko; Bracken, Adrian P.; Vernell, Richard; Moroni, M. Cristina; Christians, Fred; Grassilli, Emanuela; Prosperini, Elena; Vigo, Elena; Oliner, Jonathan D.; Helin, Kristian  
CORPORATE SOURCE: Department of Experimental Oncology, European Institute of Oncology, Milan, 20141, Italy  
SOURCE: Genes & Development (2001), 15(3), 267-285  
CODEN: GEDEEP; ISSN: 0890-9369

PUBLISHER: Cold Spring Harbor Laboratory Press  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB The retinoblastoma protein (pRB) and its two relatives, p107 and p130, regulate development and cell proliferation in part by inhibiting the activity of E2F-regulated promoters. High-d. oligonucleotide arrays were used to identify genes in which expression changed in response to activation of E2F1, E2F2, and E2F3. The E2Fs control the expression of several genes that are involved in cell proliferation. The E2Fs also regulate a number of genes involved in apoptosis, differentiation, and development. These results provide possible genetic explanations to the variety of phenotypes observed as a consequence of a deregulated pRB/E2F pathway.

REFERENCE COUNT: 61 THERE ARE 61 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 37 OF 47 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2000:628278 HCAPLUS

DOCUMENT NUMBER: 133:218538

TITLE: Cloning and cDNA sequences of human sphingosine kinase isoforms SKA, SKB and SKC and therapeutic uses

INVENTOR(S): Munroe, Donald; Gupta, Ashwani; Falzone, Germaine R.

PATENT ASSIGNEE(S): NPS Allelix Corp., Can.

SOURCE: PCT Int. Appl., 81 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000052173	A2	20000908	WO 2000-CA223	20000302
WO 2000052173	A3	20010215		

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 1999-122516P P 19990302

AB The present invention provides newly identified and isolated cDNAs and their polypeptides of the sphingosine kinase family and their uses. Three isolated polynucleotides and polypeptides for three human SK homologues are described: SKA, SKB and SKC.

L11 ANSWER 38 OF 47 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2000:84980 HCAPLUS

DOCUMENT NUMBER: 132:118362

TITLE: Protein and cDNA sequences of human kinase (clone LCBKINASE1), and uses thereof in therapy, diagnosis, and drug screening

INVENTOR(S): Duckworth, David Malcolm

PATENT ASSIGNEE(S): Smithkline Beecham P.L.C., UK

SOURCE: PCT Int. Appl., 28 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000005365	A1	20000203	WO 1999-GB2379	19990721

W: JP

RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL,  
PT, SE

EP 1098973 A1 20010516 EP 1999-934930 19990721  
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,  
IE, FI

JP 2002522017 T2 20020723 JP 2000-561311 19990721  
PRIORITY APPLN. INFO.: GB 1998-16030 A 19980722  
WO 1999-GB2379 W 19990721

AB The invention provides protein and cDNA sequences for a newly identified human protein, designated clone LCBKINASE1, which has homol. and/or structural similarity with mouse sphingosine kinase. The LCBKINASE1 gene is therefore of interest because kinases are targets of pharmaceutical intervention. In one embodiment, the invention relates to diagnostic assays for detecting diseases associated with inappropriate LCBKINASE1 activity or levels. Also disclosed are methods for utilizing LCBKINASE1 in drug screening assays and in therapy directed against diseases associated with inappropriate LCBKINASE1 activity or levels.

REFERENCE COUNT: 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 39 OF 47 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2000:788066 HCAPLUS

DOCUMENT NUMBER: 134:112107

TITLE: Expression of a catalytically inactive sphingosine kinase mutant blocks agonist-induced sphingosine kinase activation. A dominant-negative sphingosine kinase

AUTHOR(S): Pitson, Stuart M.; Moretti, Paul A. B.; Zebol, Julia R.; Xia, Pu; Gamble, Jennifer R.; Vadas, Mathew A.; D'Andrea, Richard J.; Wattenberg, Binks W.

CORPORATE SOURCE: Hanson Centre for Cancer Research, Division of Human Immunology, Institute of Medical and Veterinary Science and the Department of Medicine, University of Adelaide, Adelaide, 5000, Australia

SOURCE: Journal of Biological Chemistry (2000), 275(43), 33945-33950

CODEN: JBCHA3; ISSN: 0021-9258

PUBLISHER: American Society for Biochemistry and Molecular Biology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Sphingosine kinase (SK) catalyzes the formation of sphingosine 1-phosphate (S1P), a lipid messenger that plays an important role in a variety of mammalian cell processes, including inhibition of apoptosis and stimulation of cell proliferation. Basal levels of S1P in cells are generally low but can increase rapidly when cells are exposed to various agonists through rapid and transient activation of SK activity. To date, elucidation of the exact signaling pathways affected by these elevated S1P levels has relied on the use of SK inhibitors that are known to have direct effects on other enzymes in the cell. Furthermore, these inhibitors block basal SK activity, which is thought to have a housekeeping function in the cell. To produce a specific inhibitor of SK activation we sought to generate a catalytically inactive, dominant-neg. SK. This was accomplished by site-directed mutagenesis of Gly82 to Asp of the human SK, a residue identified through sequence similarity to the putative catalytic domain of diacylglycerol kinase. This mutant had no detectable SK activity when expressed at high levels in HEK293T cells. Activation of endogenous SK activity by tumor necrosis factor- $\alpha$  (TNF $\alpha$ ), interleukin-1 $\beta$ , and phorbol esters in HEK293T cells was blocked by expression of this inactive sphingosine kinase (hSKG82D). Basal SK activity was unaffected by expression of hSKG82D. Expression of hSKG82D had no effect on TNF $\alpha$ -induced activation of protein kinase C and sphingomyelinase activities. Thus, hSKG82D acts as a specific dominant-neg. SK to block SK activation. This discovery provides a powerful tool for the elucidation of the exact signaling pathways affected by elevated S1P levels following SK activation. To this end we have employed the dominant-neg. SK to

demonstrate that TNF $\alpha$  activation of extracellular signal-regulated kinases 1 and 2 (ERK1,2) is dependent on SK activation.

REFERENCE COUNT: 63 THERE ARE 63 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 40 OF 47 MEDLINE on STN DUPLICATE 11  
ACCESSION NUMBER: 2000387082 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 10751414  
TITLE: Molecular cloning and functional characterization of a novel mammalian sphingosine kinase type 2 isoform.  
AUTHOR: Liu H; Sugiura M; Nava V E; Edsall L C; Kono K; Poulton S; Milstien S; Kohama T; Spiegel S  
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, Georgetown University Medical Center, Washington, D. C. 20007, USA.  
CONTRACT NUMBER: GM43880 (NIGMS)  
SOURCE: Journal of biological chemistry, (2000 Jun 30) 275 (26) 19513-20.  
Journal code: 2985121R. ISSN: 0021-9258.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-AF245447; GENBANK-AF245448  
ENTRY MONTH: 200008  
ENTRY DATE: Entered STN: 20000818  
Last Updated on STN: 20000818  
Entered Medline: 20000810

AB Sphingosine-1-phosphate (SPP) has diverse biological functions acting inside cells as a second messenger to regulate proliferation and survival, and extracellularly, as a ligand for G protein-coupled receptors of the endothelial differentiation gene-1 subfamily. Based on sequence homology to murine and human sphingosine kinase-1 (SPHK1), which we recently cloned (Kohama, T., Oliver, A., Edsall, L., Nagiec, M. M., Dickson, R., and Spiegel, S. (1998) J. Biol. Chemical 273, 23722-23728), we have now cloned a second type of mouse and human sphingosine kinase (mSPHK2 and hSPHK2). mSPHK2 and hSPHK2 encode proteins of 617 and 618 amino acids, respectively, both much larger than SPHK1, and though diverging considerably, both contain the conserved domains found in all SPHK1s. Northern blot analysis revealed that SPHK2 mRNA expression had a strikingly different tissue distribution from that of SPHK1 and appeared later in embryonic development. Expression of SPHK2 in HEK 293 cells resulted in elevated SPP levels. d-erythro-dihydrosphingosine was a better substrate than d-erythro-sphingosine for SPHK2. Surprisingly, d, l-threo-dihydrosphingosine was also phosphorylated by SPHK2. In contrast to the inhibitory effects on SPHK1, high salt concentrations markedly stimulated SPHK2. Triton X-100 inhibited SPHK2 and stimulated SPHK1, whereas phosphatidylserine stimulated both type 1 and type 2 SPHK. Thus, SPHK2 is another member of a growing class of sphingolipid kinases that may have novel functions.

L11 ANSWER 41 OF 47 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN  
ACCESSION NUMBER: 2000:544738 SCISEARCH  
THE GENUINE ARTICLE: 331ZK  
TITLE: Molecular cloning and characterization of a lipid phosphohydrolase that degrades sphingosine-1-phosphate and induces cell death  
AUTHOR: Mandala S M; Thornton R; Galve-Roperh I; Poulton S; Peterson C; Olivera A; Bergstrom J; Kurtz M B; Spiegel S (Reprint)  
CORPORATE SOURCE: Georgetown Univ, Med Ctr, Dept Biochem & Mol Biol, Washington, DC 20007 USA (Reprint); Merck Res Labs, Dept Infect Dis, Rahway, NJ 07065 USA  
COUNTRY OF AUTHOR: USA  
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (5 JUL 2000) Vol. 97, No. 14,

pp. 7859-7864.

ISSN: 0027-8424.

PUBLISHER: NATL ACAD SCIENCES, 2101 CONSTITUTION AVE NW, WASHINGTON,  
DC 20418 USA.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 49  
ENTRY DATE: Entered STN: 2000  
Last Updated on STN: 2000

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Sphingosine and sphingosine-1-phosphate (SPP) are interconvertible sphingolipid metabolites with opposing effects on cell growth and apoptosis. Based on sequence homology with LBP1, a lipid phosphohydrolase that regulates the levels of phosphorylated sphingoid bases in yeast, we report here the cloning, identification, and characterization of a mammalian SPP phosphatase (mSPP1). This hydrophobic enzyme, which contains the type 2 lipid phosphohydrolase conserved sequence motif, shows substrate specificity for SPP. Partially purified Myc-tagged mSPP1 was also highly active at dephosphorylating SPP. When expressed in yeast, mSPP1 can partially substitute for the function of LBP1. Membrane fractions from human embryonic kidney HEK293 cells transfected with mSPP1 markedly degraded SPP but not lysophosphatidic acid, phosphatidic acid, or ceramide-1-phosphate. Enforced expression of mSPP1 in NIH 3T3 fibroblasts not only decreased SPP and enhanced ceramide levels, it also markedly diminished survival and induced the characteristic traits of apoptosis. Collectively, our results suggest that SPP phosphohydrolase may regulate the dynamic balance between sphingolipid metabolite levels in mammalian cells and consequently influence cell fate.

L11 ANSWER 42 OF 47 MEDLINE on STN DUPLICATE 12  
ACCESSION NUMBER: 2001301202 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 11171192  
TITLE: Characterization of sphingolipid long-chain base kinase in Arabidopsis thaliana.  
AUTHOR: Nishiura H; Tamura K; Morimoto Y; Imai H  
CORPORATE SOURCE: Department of Biology, Faculty of Science, Konan University, Kobe 658-8501, Japan.  
SOURCE: Biochemical Society transactions, (2000 Dec) 28 (6) 747-8.  
Journal code: 7506897. ISSN: 0300-5127.  
PUB. COUNTRY: England: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200105  
ENTRY DATE: Entered STN: 20010604  
Last Updated on STN: 20010604  
Entered Medline: 20010531

AB Sphingolipid long-chain base (LCB) kinase catalyses the phosphorylation of sphingolipid LCB to form LCB 1-phosphate. Based on sequence identity to a murine sphingosine kinase (murine SPHK1a), we isolated and characterized a LCB kinase-like cDNA in Arabidopsis thaliana. The deduced amino acid sequence of the homologous cDNA shows several regions that are highly conserved in LCB kinases from mouse, yeast, human and Caenorhabditis elegans. These regions are not similar to those of other known kinase families. For a functional identification, the homologous cDNA from A. thaliana was expressed in Escherichia coli, and LCB kinase activity was measured. The recombinant AtLcbk1 protein was found to utilize ATP and sphinganine. These results indicate the first identification of a gene coding for a LCB kinase in plants.

L11 ANSWER 43 OF 47 MEDLINE on STN DUPLICATE 13  
ACCESSION NUMBER: 2001097784 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 10947957  
TITLE: Human sphingosine kinase: purification, molecular cloning and characterization of the native and recombinant enzymes.  
AUTHOR: Pitson S M; D'andrea R J; Vandeleur L; Moretti P A; Xia P;

Gamble J R; Vadas M A; Wattenberg B W  
CORPORATE SOURCE: Hanson Centre for Cancer Research, Division of Human  
Immunology, Institute of Medical and Veterinary Science,  
Frome Road, Adelaide 5000, SA, Australia.  
SOURCE: Biochemical journal, (2000 Sep 1) 350 Pt 2 429-41.  
Journal code: 2984726R. ISSN: 0264-6021.  
PUB. COUNTRY: ENGLAND: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-AF200328  
ENTRY MONTH: 200102  
ENTRY DATE: Entered STN: 20010322  
Last Updated on STN: 20010322  
Entered Medline: 20010201

AB Sphingosine 1-phosphate (S1P) is a novel lipid messenger that has  
important roles in a wide variety of mammalian cellular processes  
including growth, differentiation and death. Basal levels of S1P in  
mammalian cells are generally low, but can increase rapidly and  
transiently when cells are exposed to mitogenic agents and other stimuli.  
This increase is largely due to increased activity of sphingosine  
kinase (SK), the enzyme that catalyses its formation. In the  
current study we have purified, cloned and characterized the first  
human SK to obtain a better understanding of its biochemical  
activity and possible activation mechanisms. The enzyme was purified to  
homogeneity from human placenta using ammonium sulphate  
precipitation, anion-exchange chromatography, calmodulin-affinity  
chromatography and gel-filtration chromatography. This resulted in a  
purification of over 10(6)-fold from the original placenta extract. The  
enzyme was cloned and expressed in active form in both HEK-293T cells and  
Escherichia coli, and the recombinant E. coli-derived SK purified to  
homogeneity. To establish whether post-translational modifications lead  
to activation of human SK activity we characterized both the  
purified placental enzyme and the purified recombinant SK produced in E.  
coli, where such modifications would not occur. The premise for this  
study was that post-translational modifications are likely to cause  
conformational changes in the structure of SK, which may result in  
detectable changes in the physico-chemical or catalytic properties of the  
enzyme. Thus the enzymes were characterized with respect to substrate  
specificity and kinetics, inhibition kinetics and various other  
physico-chemical properties. In all cases, both the native and  
recombinant SKs displayed remarkably similar properties, indicating that  
post-translational modifications are not required for basal activity of  
human SK.

L11 ANSWER 44 OF 47 MEDLINE on STN DUPLICATE 14  
ACCESSION NUMBER: 2000263733 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 10802064  
TITLE: Functional characterization of human  
sphingosine kinase-1.  
AUTHOR: Nava V E; Lacana E; Poulton S; Liu H; Sugiura M; Kono K;  
Milstien S; Kohama T; Spiegel S  
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology,  
Georgetown University Medical Center, 353 Basic Science  
Building, 3900 Reservoir Road NW, Washington, DC 20007,  
USA.  
CONTRACT NUMBER: GM43880 (NIGMS)  
SOURCE: FEBS letters, (2000 May 4) 473 (1) 81-4.  
Journal code: 0155157. ISSN: 0014-5793.  
PUB. COUNTRY: Netherlands  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-AF238083  
ENTRY MONTH: 200006  
ENTRY DATE: Entered STN: 20000616  
Last Updated on STN: 20000616  
Entered Medline: 20000605

AB Sphingosine kinase catalyzes the phosphorylation of

sphingosine to form sphingosine 1-phosphate (SPP), a novel lipid mediator with both intra- and extracellular functions. Based on sequence identity to murine sphingosine kinase (mSPHK1a), we cloned and characterized the first human sphingosine kinase (hSPHK1). The open reading frame of hSPHK1 encodes a 384 amino acid protein with 85% identity and 92% similarity to mSPHK1a at the amino acid level. Similar to mSPHK1a, when HEK293 cells were transfected with hSPHK1, there were marked increases in sphingosine kinase activity resulting in elevated SPP levels. hSPHK1 also specifically phosphorylated D-erythro-sphingosine and to a lesser extent sphinganine, but not other lipids, such as D,L-threo-dihydrosphingosine, N, N-dimethylsphingosine, diacylglycerol, ceramide, or phosphatidylinositol. Northern analysis revealed that hSPHK1 was widely expressed with highest levels in adult liver, kidney, heart and skeletal muscle. Thus, hSPHK1 belongs to a highly conserved unique lipid kinase family that regulates diverse biological functions.

L11 ANSWER 45 OF 47 MEDLINE on STN DUPLICATE 15  
 ACCESSION NUMBER: 2000399757 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 10847608  
 TITLE: A high-performance liquid chromatographic method to measure sphingosine 1-phosphate and related compounds from sphingosine kinase assays and other biological samples.  
 AUTHOR: Caligan T B; Peters K; Ou J; Wang E; Saba J; Merrill A H Jr  
 CORPORATE SOURCE: Department of Biochemistry, Emory University School of Medicine, Atlanta, Georgia 30322, USA.  
 CONTRACT NUMBER: CA77528-01 (NCI)  
 GM46368 (NIGMS)  
 SOURCE: Analytical biochemistry, (2000 May 15) 281 (1) 36-44.  
 Journal code: 0370535. ISSN: 0003-2697.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200008  
 ENTRY DATE: Entered STN: 20000824  
 Last Updated on STN: 20000824  
 Entered Medline: 20000816

AB Sphingosine 1-phosphate is an intermediate of sphingosine catabolism as well as a potent signaling compound. Conditions were established for the extraction and analysis of sphingosine 1-phosphate and other sphingoid base 1-phosphates from in vitro sphingosine kinase assays and other biological samples. The sphingoid base 1-phosphates were extracted in high yield (85%) using small C-18 reverse-phase columns (LiChroprep RP-18). After the extracts were treated with 0.1 N KOH to remove glycerolipids, the sphingoid base 1-phosphates were converted to fluorescent o-phthalaldehyde derivatives that were separated by HPLC using C-18 columns with a mobile phase of methanol:10 mM potassium phosphate (pH 7.2):1 M tetrabutylammonium dihydrogen phosphate (in water) (83:16:1, v/v/v). The o-phthalaldehyde derivative of sphingosine 1-phosphate was reasonably stable ( $t(1/2) > \text{or} = 18 \text{ h}$ ) when EDTA was present and could be detected in picomole amounts. The HPLC retention time of the sphingoid base 1-phosphates could be shifted by adjusting the mobile phase to pH 5.5, which is useful in separating overlapping compounds (such as sphingosine 1-phosphate and 4-D-hydroxysphinganine) and in confirming the identity of sphingoid base 1-phosphates in biological samples. The extraction procedure and HPLC method facilitated assays of sphingosine kinase with different sphingoid bases as substrates and/or inhibitors and enabled the quantitation of sphingoid base 1-phosphates in human plasma, serum, and platelets as well as in strains of *Saccharomyces cerevisiae* with mutations in sphingolipid metabolism.

L11 ANSWER 46 OF 47 MEDLINE on STN DUPLICATE 16  
 ACCESSION NUMBER: 2000323213 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 10863092  
 TITLE: Human sphingosine kinase:



molecular cloning, functional characterization and tissue distribution.

AUTHOR: Melendez A J; Carlos-Dias E; Gosink M; Allen J M; Takacs L  
CORPORATE SOURCE: Department of Molecular and Cellular Biology, Institut de  
Recherche Jouveinal/Parke-Davis, Fresnes, France..  
alirio.melendez@wl.com  
SOURCE: Gene, (2000 Jun 13) 251 (1) 19-26.  
Journal code: 7706761. ISSN: 0378-1119.  
PUB. COUNTRY: Netherlands  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200007  
ENTRY DATE: Entered STN: 20000810  
Last Updated on STN: 20000810  
Entered Medline: 20000727

AB Sphingosine-1-phosphate (SPP), the product of **sphingosine kinase**, is an important signaling molecule with intra- and extracellular functions. The cDNA for the mouse **sphingosine kinase** has recently been reported. In this paper we describe the cloning, expression and characterization of the human **sphingosine kinase** (huSPHK1). Sequence analysis comparison revealed that this kinase is evolutionarily very conserved, having a high degree of **homology** with the murine enzyme, and presenting several conserved regions with bacteria, yeast, plant, and mammalian proteins. Expressed huSPHK1 cDNA specifically phosphorylates D-erythro-sphingosine and, to a lesser extent, D, L-erythro-dihydrosphingosine, and not at all the 'threo' isoforms of dihydrosphingosine; hydroxy-ceramide or non-hydroxy-ceramide; diacylglycerol (DAG); phosphatidylinositol (PI); phosphatidylinositol-4-phosphate (PIP); or phosphatidylinositol-4, 5-bisphosphate (PIP(2)). huSPHK1 shows typical Michaelis-Menten kinetics ( $V_{max}=56\mu\text{M}$  and  $K_m=5\mu\text{M}$ ). The kinase is inhibited by D,L-threo-dihydrosphingosine ( $K_i=3\mu\text{M}$ ), and by N, N-dimethyl-sphingosine ( $K_i=5\mu\text{M}$ ). Northern blots indicate highest expression in adult lung and spleen, followed by peripheral blood leukocyte, thymus and kidney, respectively. It is also expressed in brain and heart. In addition, database searches with the stSG2854 sequence indicate that huSPHK1 is also expressed in endothelial cells, retinal pigment epithelium, and senescent fibroblasts.

L11 ANSWER 47 OF 47 MEDLINE on STN  
ACCESSION NUMBER: 1998395082 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 9726979  
TITLE: Molecular cloning and functional characterization of murine **sphingosine kinase**.  
AUTHOR: Kohama T; Olivera A; Edsall L; Nagiec M M; Dickson R; Spiegel S  
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, Georgetown University Medical Center, Washington, DC 20007, USA.  
CONTRACT NUMBER: CA61774 (NCI)  
GM43880 (NIGMS)  
SOURCE: Journal of biological chemistry, (1998 Sep 11) 273 (37) 23722-8.  
Journal code: 2985121R. ISSN: 0021-9258.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-AF068748; GENBANK-AF068749  
ENTRY MONTH: 199810  
ENTRY DATE: Entered STN: 19981021  
Last Updated on STN: 19981021  
Entered Medline: 19981013

AB Sphingosine-1-phosphate (SPP) is a novel lipid messenger that has dual function. Intracellularly it regulates proliferation and survival, and extracellularly, it is a ligand for the G protein-coupled receptor Edg-1. Based on peptide sequences obtained from purified rat kidney **sphingosine kinase**, the enzyme that regulates SPP

levels, we report here the cloning, identification, and characterization of the first mammalian sphingosine kinases (murine SPHK1a and SPHK1b). Sequence analysis indicates that these are novel kinases, which are not similar to other known kinases, and that they are evolutionarily conserved. Comparison with *Saccharomyces cerevisiae* and *Caenorhabditis elegans* sphingosine kinase sequences shows that several blocks are highly conserved in all of these sequences. One of these blocks contains an invariant, positively charged motif, GGKGGK, which may be part of the ATP binding site. From Northern blot analysis of multiple mouse tissues, we observed that expression was highest in adult lung and spleen, with barely detectable levels in skeletal muscle and liver. Human embryonic kidney cells and NIH 3T3 fibroblasts transiently transfected with either sphingosine kinase expression vectors had marked increases (more than 100-fold) in sphingosine kinase activity. The enzyme specifically phosphorylated D-erythro-sphingosine and did not catalyze the phosphorylation of phosphatidylinositol, diacylglycerol, ceramide, D,L-threo-dihydrosphingosine or N, N-dimethylsphingosine. The latter two sphingolipids were competitive inhibitors of sphingosine kinase in the transfected cells as was previously found with the purified rat kidney enzyme. Transfected cells also had a marked increase in mass levels of SPP with a concomitant decrease in levels of sphingosine and, to a lesser extent, in ceramide levels. Our data suggest that sphingosine kinase is a prototypical member of a new class of lipid kinases. Cloning of sphingosine kinase is an important step in corroborating the intracellular role of SPP as a second messenger.

=> d his

(FILE 'HOME' ENTERED AT 12:22:25 ON 13 SEP 2005)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 12:22:51 ON 13 SEP 2005

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L1      181 S CERAMIDE (W)KINASE?
L2      99 S HUMAN AND L1
L3      7258991 S CLON? OR EXPRESS? OR RECOMBINANT
L4      49 S L2 AND L3
L5      22 DUP REM L4 (27 DUPLICATES REMOVED)
L6      2 S SHINGOSINE (W)KINASE?
L7      2026 S SPHINGOSINE (W)KINASE?
L8      1467318 S HOMOLOG? OR IDENTIT?
L9      526525 S HUMAN AND L8
L10     99 S L7 AND L9
L11     47 DUP REM L10 (52 DUPLICATES REMOVED)
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=> e kossida s/au

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E1      2      KOSSICK R/AU
E2      2      KOSSICK THOMAS/AU
E3      39 --> KOSSIDA S/AU
E4      33      KOSSIDA SOPHIA/AU
E5      1      KOSSIDA T/AU
E6      1      KOSSIDAS A T/AU
E7      1      KOSSIDAS C/AU
E8      7      KOSSIEN I/AU
E9      2      KOSSIEN IRMTRUD/AU
E10     12      KOSSIG J/AU
E11     1      KOSSIGAN K/AU
E12     8      KOSSII IU E/AU
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=> s e3-e4

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L12     72 ("KOSSIDA S"/AU OR "KOSSIDA SOPHIA"/AU)
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=> e enicas j/au

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E2      1      ENICA S/AU
E3      0 --> ENICAS J/AU
E4      7      ENICE F/AU
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E6	1	ENICHEK V/AU
E7	1	ENICHEN F/AU
E8	11	ENICHEN W A/AU
E9	1	ENICHEN WILLIAM/AU
E10	2	ENICHEN WILLIAM A/AU
E11	10	ENICHEV V M/AU
E12	1	ENICHIMAIR H/AU

=> e encinas j/au

E1	1	ENCINAS GRANDES ANTONIO LUIS/AU
E2	7	ENCINAS I P/AU
E3	27 -->	ENCINAS J/AU
E4	42	ENCINAS J A/AU
E5	6	ENCINAS J C/AU
E6	1	ENCINAS J E/AU
E7	6	ENCINAS J I/AU
E8	34	ENCINAS J L/AU
E9	47	ENCINAS J M/AU
E10	21	ENCINAS J P/AU
E11	2	ENCINAS J R/AU
E12	1	ENCINAS JAVIER/AU

=> s e3

L13 27 "ENCINAS J"/AU

=> e takao e/au

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E2	8	TAKAO DAISUKE/AU
E3	19 -->	TAKAO E/AU
E4	1	TAKAO EDAHIRO/AU
E5	1	TAKAO EIKICHI/AU
E6	7	TAKAO EIKO/AU
E7	1	TAKAO EISUKE/AU
E8	2	TAKAO EMI/AU
E9	1	TAKAO ENOMOTO/AU
E10	3	TAKAO ETSUKO/AU
E11	81	TAKAO F/AU
E12	1	TAKAO FUJIKAZU/AU

=> s e3

L14 19 "TAKAO E"/AU

=> d his

(FILE 'HOME' ENTERED AT 12:22:25 ON 13 SEP 2005)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 12:22:51 ON 13 SEP 2005

L1	181	S CERAMIDE (W)KINASE?
L2	99	S HUMAN AND L1
L3	7258991	S CLON? OR EXPRESS? OR RECOMBINANT
L4	49	S L2 AND L3
L5	22	DUP REM L4 (27 DUPLICATES REMOVED)
L6	2	S SHINGOSINE (W)KINASE?
L7	2026	S SPHINGOSINE (W)KINASE?
L8	1467318	S HOMOLOG? OR IDENTIT?
L9	526525	S HUMAN AND L8
L10	99	S L7 AND L9
L11	47	DUP REM L10 (52 DUPLICATES REMOVED)
		E KOSSIDA S/AU
L12	72	S E3-E4
		E ENICAS J/AU
		E ENCINAS J/AU
L13	27	S E3
		E TAKAO E/AU
L14	19	S E3

=> s l12 or l13 or l14

L15 117 L12 OR L13 OR L14

=> s l2 and l15

L16 1 L2 AND L15

=> d all

L16 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 2004:802537 HCAPLUS

DN 141:289087

ED Entered STN: 01 Oct 2004

TI Expression and screening for compounds regulating activity of  
**ceramide kinase** in tissues, for use in treatment of  
human diseases

IN Kossida, Sophia; Encinas, Jeffrey; Takao, Eiko

PA Bayer Aktiengesellschaft, Germany

SO U.S. Pat. Appl. Publ., 50 pp., Cont.-in-part of U.S. Ser. No. 969,896,  
abandoned.

CODEN: USXXCO

DT Patent

LA English

IC ICM A61K038-17

ICS C07H021-04; C12N009-12

INCL 514002000; 435069100; 435194000; 435320100; 435325000; 536023200

CC 1-12 (Pharmacology)

Section cross-reference(s): 3, 7, 14

FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 2004192580	A1	20040930	US 2003-631958	20031219
	US 2003125533	A1	20030703	US 2001-969896	20011004
PRAI	US 2000-238005P	P	20001006		
	US 2001-314113P	P	20010823		
	US 2001-969896	B2	20011004		

CLASS

PATENT NO.	CLASS	PATENT FAMILY CLASSIFICATION CODES
US 2004192580	ICM	A61K038-17
	ICS	C07H021-04; C12N009-12
	INCL	514002000; 435069100; 435194000; 435320100; 435325000; 536023200
US 2004192580	NCL	514/002.000
	ECLA	C12N009/12B1
US 2003125533	NCL	536/023.100
	ECLA	C12N009/12B1

AB This invention relates to expression and screening for compds. regulating activity of **ceramide kinase** in tissues, for use in treatment of human diseases. **Ceramide kinase** cDNA and protein sequences, as well as expression profiles in various human tissues and cell lines, are provided. Reagents that regulate human **ceramide kinase** protein activity and reagents that bind to human **ceramide kinase** gene products can be used to regulate intracellular signaling and consequently cell proliferation and apoptosis. Methods of drug screening for reagents influencing **ceramide kinase** activity in HEK293 cells was exemplified by use of sphingosine derivs., in conjunction with anal. of cellular apoptotic response. Such regulation is particularly useful for treating allergies including but not limited to asthma, autoimmune diseases such as rheumatoid arthritis, inflammatory disease, transplant rejection, and cancer, particularly lymphocytic leukemias, and could be a useful target of vaccination enhancing adjuvants. Central and peripheral nervous system disorders, such as Parkinson's disease, also can be treated.

ST human **ceramide kinase** cDNA sequence;  
**ceramide kinase** expression human tissue  
distribution; drug screening **ceramide kinase**  
regulation apoptosis human disease therapy

IT Animal cell line

(Hek 293; expression and screening for compds. regulating activity of

ceramide kinase in tissues, for use in treatment of human diseases)

IT Animal cell line  
(IMR-90; expression and screening for compds. regulating activity of ceramide kinase in tissues, for use in treatment of human diseases)

IT Animal cell line  
(JURKAT; expression and screening for compds. regulating activity of ceramide kinase in tissues, for use in treatment of human diseases)

IT Animal cell line  
(Ramos; expression and screening for compds. regulating activity of ceramide kinase in tissues, for use in treatment of human diseases)

IT HeLa cell  
(S3; expression and screening for compds. regulating activity of ceramide kinase in tissues, for use in treatment of human diseases)

IT Animal cell line  
(U937; expression and screening for compds. regulating activity of ceramide kinase in tissues, for use in treatment of human diseases)

IT Antisense oligonucleotides  
RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(against ceramide kinase gene; expression and screening for compds. regulating activity of ceramide kinase in tissues, for use in treatment of human diseases)

IT Antibodies and Immunoglobulins  
RL: ARG (Analytical reagent use); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(against ceramide kinase; expression and screening for compds. regulating activity of ceramide kinase in tissues, for use in treatment of human diseases)

IT Molecular association  
(between ceramide kinase and reagents; expression and screening for compds. regulating activity of ceramide kinase in tissues, for use in treatment of human diseases)

IT Epithelium  
(bronchial; expression and screening for compds. regulating activity of ceramide kinase in tissues, for use in treatment of human diseases)

IT Nervous system, disease  
(central, inhibition of; expression and screening for compds. regulating activity of ceramide kinase in tissues, for use in treatment of human diseases)

IT Fusion proteins (chimeric proteins)  
RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(ceramide kinase fusion with GST; expression and screening for compds. regulating activity of ceramide kinase in tissues, for use in treatment of human diseases)

IT Brain  
(cerebellum; expression and screening for compds. regulating activity of ceramide kinase in tissues, for use in treatment of human diseases)

IT Intestine  
(colon; expression and screening for compds. regulating activity of ceramide kinase in tissues, for use in treatment of human diseases)

IT Nucleic acid amplification (method)  
Nucleic acid hybridization  
(detection of ceramide kinase gene; expression and screening for compds. regulating activity of ceramide kinase in tissues, for use in treatment of human diseases)

IT Cell  
(detection of reagent association with ceramide kinase

in cell or cell-free system; expression and screening for compds.  
regulating activity of **ceramide kinase** in tissues,  
for use in treatment of human diseases)

- IT Bronchi  
Trachea (anatomical)  
(epithelium; expression and screening for compds. regulating activity  
of **ceramide kinase** in tissues, for use in treatment  
of human diseases)
- IT Adrenal gland  
Apoptosis  
B cell (lymphocyte)  
Bone marrow  
Drug screening  
Endothelium  
Fluorescent indicators  
Heart  
Human  
Kidney  
Leukocyte  
Lymph node  
Mammary gland  
Molecular cloning  
Monocyte  
Mononuclear cell (leukocyte)  
Neutrophil  
Pancreas  
Placenta  
Prostate gland  
Salivary gland  
Spinal cord  
Spleen  
Stomach  
T cell (lymphocyte)  
Testis  
Thymus gland  
Thyroid gland  
Tonsil  
Trachea (anatomical)  
Uterus  
(expression and screening for compds. regulating activity of  
**ceramide kinase** in tissues, for use in treatment of  
human diseases)
- IT Sulfatides  
cDNA  
RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(expression and screening for compds. regulating activity of  
**ceramide kinase** in tissues, for use in treatment of  
human diseases)
- IT Brain  
Liver  
(fetal or adult; expression and screening for compds. regulating  
activity of **ceramide kinase** in tissues, for use in  
treatment of human diseases)
- IT Lung  
(fibroblast; expression and screening for compds. regulating activity  
of **ceramide kinase** in tissues, for use in treatment  
of human diseases)
- IT Gene, animal  
RL: ANT (Analyte); BSU (Biological study, unclassified); ANST (Analytical  
study); BIOL (Biological study)  
(for **ceramide kinase**, expression profile of;  
expression and screening for compds. regulating activity of  
**ceramide kinase** in tissues, for use in treatment of  
human diseases)
- IT Test kits  
(for detection of **ceramide kinase** gene; expression  
and screening for compds. regulating activity of **ceramide**  
**kinase** in tissues, for use in treatment of human  
diseases)

IT cDNA sequences  
(for human ceramide kinase gene;  
expression and screening for compds. regulating activity of  
ceramide kinase in tissues, for use in treatment of  
human diseases)

IT Protein sequences  
(for human ceramide kinase; expression  
and screening for compds. regulating activity of ceramide  
kinase in tissues, for use in treatment of human  
diseases)

IT T cell (lymphocyte)  
(helper cell/inducer, TH1; expression and screening for compds.  
regulating activity of ceramide kinase in tissues,  
for use in treatment of human diseases)

IT T cell (lymphocyte)  
(helper cell/inducer, TH2; expression and screening for compds.  
regulating activity of ceramide kinase in tissues,  
for use in treatment of human diseases)

IT Allergy  
Autoimmune disease  
Neoplasm  
(inhibition of; expression and screening for compds. regulating  
activity of ceramide kinase in tissues, for use in  
treatment of human diseases)

IT Fibroblast  
(lung; expression and screening for compds. regulating activity of  
ceramide kinase in tissues, for use in treatment of  
human diseases)

IT RNA  
Ribozymes  
RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(promoting decreased ceramide kinase activity;  
expression and screening for compds. regulating activity of  
ceramide kinase in tissues, for use in treatment of  
human diseases)

IT PCR (polymerase chain reaction)  
(real-time; expression and screening for compds. regulating activity of  
ceramide kinase in tissues, for use in treatment of  
human diseases)

IT Muscle  
(skeletal; expression and screening for compds. regulating activity of  
ceramide kinase in tissues, for use in treatment of  
human diseases)

IT Intestine  
(small; expression and screening for compds. regulating activity of  
ceramide kinase in tissues, for use in treatment of  
human diseases)

IT Muscle  
(smooth, bronchial or tracheal; expression and screening for compds.  
regulating activity of ceramide kinase in tissues,  
for use in treatment of human diseases)

IT Therapy  
(targeting ceramide kinase expression or activity;  
expression and screening for compds. regulating activity of  
ceramide kinase in tissues, for use in treatment of  
human diseases)

IT Epithelium  
(tracheal; expression and screening for compds. regulating activity of  
ceramide kinase in tissues, for use in treatment of  
human diseases)

IT 762415-26-9 762415-28-1 762415-29-2  
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL  
(Biological study)  
(amino acid sequence; expression and screening for compds. regulating  
activity of ceramide kinase in tissues, for use in  
treatment of human diseases)

IT 50812-37-8, Glutathione S transferase  
RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(ceramide kinase fusion with GST; expression and

screening for compds. regulating activity of **ceramide kinase** in tissues, for use in treatment of human diseases)

- IT 123175-68-8P, **Ceramide kinase**  
RL: BSU (Biological study, unclassified); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation)  
(containing degenerate code, fragment, derivative, allele; expression and screening for compds. regulating activity of **ceramide kinase** in tissues, for use in treatment of human diseases)
- IT 123-78-4, Sphingosine 764-22-7, Sphinganine 3102-57-6, C2 Ceramide 74713-59-0, C8 Ceramide  
RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(expression and screening for compds. regulating activity of **ceramide kinase** in tissues, for use in treatment of human diseases)
- IT 392127-42-3, GENBANK AF245447  
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)  
(expression and screening for compds. regulating activity of **ceramide kinase** in tissues, for use in treatment of human diseases)
- IT 762415-25-8 762415-27-0  
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)  
(nucleotide sequence; expression and screening for compds. regulating activity of **ceramide kinase** in tissues, for use in treatment of human diseases)
- IT 762418-53-1 762418-54-2 762418-55-3 762418-56-4 762418-57-5  
762418-58-6 762418-59-7 762418-60-0 762418-61-1  
RL: PRP (Properties)  
(unclaimed nucleotide sequence; expression and screening for compds. regulating activity of **ceramide kinase** in tissues, for use in treatment of human diseases)
- IT 762418-52-0  
RL: PRP (Properties)  
(unclaimed protein sequence; expression and screening for compds. regulating activity of **ceramide kinase** in tissues, for use in treatment of human diseases)

=> d his

(FILE 'HOME' ENTERED AT 12:22:25 ON 13 SEP 2005)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 12:22:51 ON 13 SEP 2005

- L1 181 S CERAMIDE (W) KINASE?  
L2 99 S HUMAN AND L1  
L3 7258991 S CLON? OR EXPRESS? OR RECOMBINANT  
L4 49 S L2 AND L3  
L5 22 DUP REM L4 (27 DUPLICATES REMOVED)  
L6 2 S SHINGOSINE (W) KINASE?  
L7 2026 S SPHINGOSINE (W) KINASE?  
L8 1467318 S HOMOLOG? OR IDENTIT?  
L9 526525 S HUMAN AND L8  
L10 99 S L7 AND L9  
L11 47 DUP REM L10 (52 DUPLICATES REMOVED)  
E KOSSIDA S/AU  
L12 72 S E3-E4  
E ENICAS J/AU  
E ENCINAS J/AU  
L13 27 S E3  
E TAKAO E/AU  
L14 19 S E3  
L15 117 S L12 OR L13 OR L14  
L16 1 S L2 AND L15



	L #	Hits	Search Text
1	L1	52	ceramide adj kinase\$2
2	L2	7676 95	clon\$3 or express\$3 or recombinant
3	L3	5	l1 same l2
4	L4	7	sphingosine adj kinase\$2
5	L5	2280 49	homolog\$3 or identit\$3
6	L6	2	l4 same l5
7	L7	1466 0	KOSSIDA KOSSIDA- SOPHIA ENCINAS ENCINAS-JEFFREY TAKAO
8	L8	1	l1 and l7

	Issue Date	Pages	Document ID	Title
1	20040930	50	US 20040192580 A1	Regulation of human ceramide kinase
2	20040701	101	US 20040126834 A1	Compositions and methods for the modulation of sphingolipid metabolism and/or signaling
3	20031127	81	US 20030219782 A1	Compositions and methods for the modulation of sphingolipid metabolism and/or signaling
4	20030828	22	US 20030162206 A1	Ceramide kinase and DNA encoding it
5	20021015	34	US 6465618 B1	Mitogen activated protein kinase (MAPK) kinase

	<b>Issue Date</b>	<b>Page s</b>	<b>Document ID</b>	<b>Title</b>
<b>1</b>	20040930	50	US 2004019258 0 A1	Regulation of human ceramide kinase
<b>2</b>	20030703	47	US 2003012553 3 A1	Regulation of human sphingosine kinase- like protein

	Issue Date	Page s	Document ID	Title
1	20040930	50	US 2004019258 0 A1	Regulation of human ceramide kinase